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Conversion of microalgae to biofuel

Emma Suali, Rosalam Sarbatly*

School of Engineering and IT, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

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ABSTRACT

This paper primarily presents an overall review of the use of microalgae as a biofuel feedstock. Among the microalgae that have potential as biofuel feedstock, *Chlorella*, specifically, was thoroughly discussed because of its ability to adapt both to heterotrophic and phototrophic culture conditions. The lipid content and biomass productivity of microalgae can be up to 80% and 7.3 g/l/d based on the dried weight of biomass, respectively, making microalgae an ideal candidate as a biofuel feedstock. The set-up of the system and the biomass productivity of microalgae cultivated in an open pond and a photobioreactor were also compared in this work. The effect of the culture condition is discussed based on the two-stage culture period. The issues that were discussed include the light condition and the CO_2, DO and N supply. The microalgal productivities under heterotrophic and phototrophic culture conditions were also compared and highlighted in this work. The harvesting process and type of flocculants used to aid the harvesting were highlighted by considering the final yield of biomass. A new idea regarding how to harvest microalgae based on positive and negative charges was also proposed in this work. The extraction methods and solvents discussed were primarily for the conventional and newly invented techniques. Conversion processes such as transesterification and thermochemical processes were discussed, sketched in figures and summarized in tables. The cost–benefit analysis of heterotrophic culture and the cultivation system was highlighted at the end of this work. Other benefits of microalgae are also mentioned in this work to give further support for the use of microalgae as a feedstock for biofuel production.

Contents

* Corresponding author. Tel.: +60 88 320000x3990/3445; fax: +60 88 88320348.
E-mail addresses: emma.suali@gmail.com (E. Suali), rslam@ums.edu.my (R. Sarbatly).

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1. Background

The global primary energy consumption, including oil, natural gas, nuclear and coal, has declined approximately 1.1% in 2009 [1]. However, the production of oil and natural gas also declined approximately 7.3 and 2.1%, respectively, showing that world energy source is reducing. Because of this concern, sustainable energy sources such as biofuels are important. There are many biomasses that have been proposed as biofuel feedstocks such as palm oil, jatropha and microalgae. Among these biomasses, microalgae have received notable attention because of their high photosynthetic rate, which can be more than 6.9×10^4 cells/ml/h. This average estimate was based on the *Chlorella vulgaris* cell number: 5.7×10^7 cells/ml for 34 days in control media [2]. This result shows that microalgae have a photosynthetic rate that is approximately 50 times higher compared to terrestrial plants.

Microalgae have been reported to amass more than 70% lipid on a dry weight basis [3]. However, the production cost of microalgae as a biofuel feedstock was higher compared to the final yield of the product. Despite this issue, microalgae remains imperative as a future energy feedstock, as it requires less land compared to other commercial crops such as palm oil or jatropha [4,5].

The upstream impacts of microalgal cultivation such as the demand for carbon dioxide (CO_2) can be reduced by using flue gas [6], which in turn favors the reduction of environmental issues such as global warming. In addition, microalgae are rich in biochemical compounds such as docosahexaenoic acid (DHA) and astaxanthin, which can be used for various medical purposes and have elicited increasing interest in advanced research. Chemical compounds of microalgae also can be incorporated into cosmetics and used as a source for valuable molecules such as polyunsaturated fatty acid oils, which can be added to infant formulas and nutritional supplements [7]. Based on these advantages, it is desirable to increase the lipid content in microalgae as much of these chemical compounds lie within the lipid cell.

The lipid content of microalgae, specifically the triglyceride content, is important for biodiesel. Biomass, however, can be converted into biofuel oil through a thermochemical conversion process. Thus, this work presents an overall review on the use of microalgae as a biofuel feedstock, specifically on the culture, harvesting, extraction and conversion processes that could increase the lipid and biomass productivity of microalgae. The economic evaluations are also thoroughly discussed in this work.

2. Microalgae as a biofuel feedstock

2.1. Introduction

The success of biodiesel production, which is one type of biofuel, from microalgae depends on the content of TAG (triglyceride), which composed more than 70% of the lipid content [8,9] and the biomass productivity. High TAG in the lipid content is most likely the main reason that most studies reported in the literature refer to lipid instead of TAG as the feedstock for the biodiesel production. Based on the transesterification theory, the ratio of the feedstock mass and biodiesel produced is 1:1, which means the lower the

mass of the feedstock, the lower amount of biodiesel produced. A review analysis conducted by Sun et al. [10] showed that the algal oil production costs $10.87–13.32 per gallon (in US Dollars), whereas the cost of triglyceride production is $3 per gallon. One of the factors that affect microalgal productivity is genetics. Thus, the selection of microalgae for cultivation is important. Therefore, this work assessed the microalgal productivity and culture conditions that are suitable for cultivation purposes.

Lipids comprise a fraction of biomass that represents the triglycerides formed as the energy storage for microalgae. In general, TAG is a glycerol esterified with three fatty acids, and in the presents of alcohol it reacts to form biodiesel with glycerol as a by-product. However, biomass yield provides a measure of efficiency of the photosynthesis and cultivation procedures. Thus, the biomass yield affects the biodiesel production cost. In addition, selecting a suitable microalgal species for cultivation purposes while conserving the high lipid contents and growth rates could support the eco-design of an efficient and sustainable production chain [11].

The transformation of microalgae into biofuel is shown in Fig. 1. As shown, the first stage of conversion of microalgae to biofuel is a cultivation process. Microalgae can be cultivated in either a photobioreactor or an open system such as raceway pond. Microalgal cells are then harvested using either centrifugation or filtration with the aid of flocculants. The harvested biomass is either extracted to obtain its oil or converted into biodiesel through biochemical conversion. The high heating value of microalgal biomass, which is 24 MJ/kg [6], makes it suitable for the production of bio oil fuel, syngas and other products.

2.2. Microalgal lipid and biomass productions

Genetics is one of the factors that affect the microalgal lipid and biomass productivity. Thus, it is important to choose species that have the potential for commercialization. Because of this concern, Table 1 was prepared to compare the biomass and lipid production of well-known microalgae quoted from various literatures from the 2000s. Table 2 shows a comparison of culture techniques of the microalgae listed in Table 1. Table 1 shows that *Chlorella* achieved the highest average lipid and biomass yield among the tested algae, specifically *C. emersonii* and *C. protothecoides.* Although *C. vulgaris* and *C. minutissima* are capable of producing high lipids content, the triglycerides content is low, thus these species are inefficient to as biodiesel feedstocks if they are intended for use alone [12]. As listed in Table 1, the *Nannochloropsis* species are capable in producing up to 60% lipid content. In addition, the biomass yield is approximately 0.48 g/l/d if aerated with 2% CO_2 [13,14]. This shows that *Nannochloropsis* species have a high potential to be used as biofuel feedstock. However, the average lipid content and biomass yield of this alga is lower than those of *Schizochytrium limacinum*, *C. emersonii* and *C. protothecoides*.

The lipid content of *Chaetoceros calcitrans*, *Neochloris oleoabundans* and *Scenedesmus obliquus* are among the lowest that have been reported, as listed in Table 1 [15,16]. Even after cultivated under nitrogen starvation conditions, the lipid content of *N. oleoabundans* was only 37% on a dry weight basis [17]. As shown in Table 1, the biomass yield is in the range of 0.05–0.09 g/l/d. These results show that these algae are unproductive as biofuel feedstocks in their current state. Tables 1 and 2 also show that phototrophic and heterotrophic culture conditions would result in different biomass and lipid yields for the same microalgal strain [19,20].

2.3. Overview on some selected microalgal species

Table 1 shows that the current trends of microalgal species for lipid production favor *Chlorella*, specifically *C. emersonii*, *C. minutissima*, *C. vulgaris* and *C. protothecoides*. *Chlorella* spp. were reported to be capable of producing more than 63% lipid content on a dry biomass basis [21]. In addition, *Chlorella* species were also reported to have high flexibility to adapt to diverse culture conditions and are likely the largest strain among microalgal species that have been tested as a biofuel feedstock that can be cultivated under phototrophic and heterotrophic [18,19].

Chlorella is a single-celled green alga and is ellipsoidal in its shape with a diameter range from 3 to 12 μm. The properties of the bio oil produced by heterotrophic *C. protothecoides* are comparable to fossil oil, making it suitable as a biofuel feedstock [22,23]. Heterotrophic *C. protothecoides* is composed of 40–60% lipid, 10–28% protein, 11–15% carbohydrate and 6% ash [19,22,23]. The fatty acid content is mainly composed of oleic, linoleic, palmitic and stearic acids [24,25]. However, the composition of *C. emersonii* is 23–63% lipid, 36% protein and 41% carbohydrate [21]. These results show that *Chlorella* species are suitable as candidates for biofuel production. The biodiesel produced from this species were acid methyl ester, linoleic acid methyl ester and oleic acid methyl ester [26]. Unsaturated fatty acids methyl ester comprised over 82% of the total biodiesel content [19,20]. Therefore, the properties of the biodiesel produced from *Chlorella* comply with ASTM 6751, the US Standard for biodiesel [27]. In addition to biodiesel, some microalgae were also reported as good producers of hydrogen [28,29]. Hydrogen is high in energy, and an engine fueled by pure hydrogen produces almost no pollution. Furthermore, microalgal biomass is considered to be a renewable and green method of producing energy. Moreover, an industrial company that produces biofuel from microalgal biomass has confirmed there is no toxic waste from their system [30]. This shows that microalgae have high potential as an environment-friendly energy source that could produce a variety products for biofuel.

3. Culture methods for increasing the productivity of microalgae

3.1. Introduction

Based on the energy balance of the biofuel production from microalgae, at least 25% of the recoverable energy yield depends on the cultivation approach. By manipulating the cultivation method, such as by adding enough or inadequate nitrate for a certain period, microalgae with high lipid contents can be produced. The cost of the conversion process can be further reduced through the addition of suitable chemicals or the application of suitable technologies. Compared to other biodiesel crops such as palm oil and jatropha, microalgae require specific techniques for cultivation, harvesting, extraction and conversion for the process to be economically feasible. Although microalgae were reported to produce high lipid contents compared with other crops, the capital costs to transform microalgal biomass into biofuel are high [31,32]. This problem has hindered the successful commercialization of algal biofuel as an energy source to reduce the consumption of fossil fuel.

One way to make microalgae feasible is by increasing the lipid production to recover the overall production cost. The effort to increase lipid production can be classified into two techniques: the manipulation of the culture system in terms of the engineering and design, and changing the phycological metabolism of the microalgal cell. Both techniques could increase the biomass and lipid production rate by more than 10-fold. One phycological approach, the so-called two-stage growth period, can be manipulated to increase the lipid production.

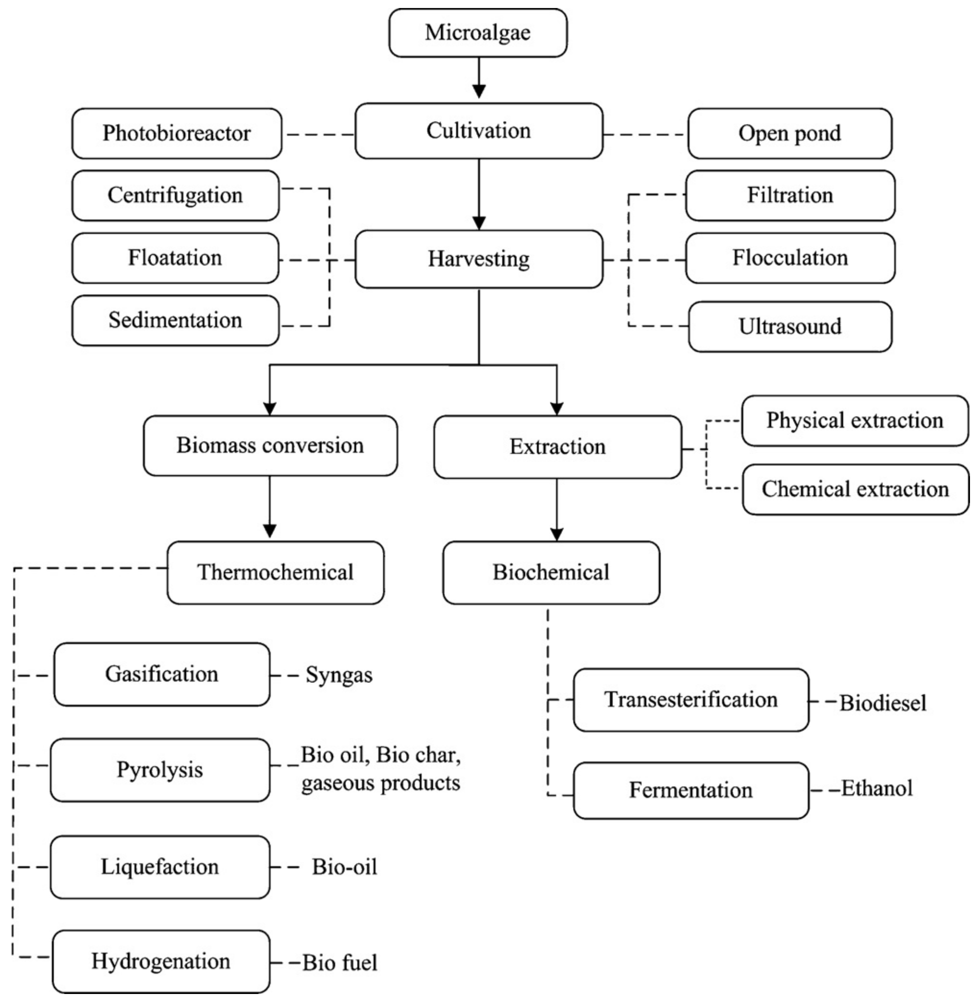


Fig. 1. A summary of microalgal transformations into biofuel. The main product of each process is shown at the end of the transformation route.

3.2. Two-stage culture of microalgae

The two-stage culture of microalgae is a culturing process that manipulates the culture conditions and nutrient feed in terms of the period and concentration to increase the cell reproduction rate and lipid content of the microalgae. The first stage is the development of the cell numbers during the zoospore settlement, and the second stage is an increase in the lipid content during synchronous growth. The synchronous growth of microalgae can be defined as a process where the cell number or zoospore of the microalgae is increased while also increasing their size. However, during the second stage, more attention is given to how to enrich each cell with lipids rather than increasing the cell number. This idea based on microalgal nature, which actively responds to nutrient starvation or nutrient excess, including culture conditions.

3.2.1. Effect of the dissolved oxygen

It is well-known that dissolved oxygen (DO) is lethal to microalgal cells. However, the DO levels can be manipulated to achieve two-stage growth by controlling the concentration and dispersion rate in the culture medium. The effect of DO in the culture medium suggests that two mechanisms exist within the cell to protect the oxygen-sensitive components of nitrogenase enzyme. The nitrogenase enzyme is important in fixing atmospheric nitrogen to be used as a nutrient feed to microalgal growth. The mechanism involves augmented respiration to scavenge the excess oxygen and a conformational state of nitrogenase that prevents damage by oxygen. By allowing a DO concentration of approximately 50% in the culture medium, the development of the cell numbers during the zoospore settlement could be enhanced [33]. The release of zoospores can be counted under the microscope, and the count can be used as a base analysis to determine the suitable period for the optimum cell number development in microalgae. Because the effect of DO is dependent on the microalgal species, it is difficult to state the optimum period that is required before there is a decline in the cell number development. The second stage can be achieved by allowing a DO concentration of approximately 10% in the culture medium with a media renewable rate between 10 and 40% [34], as a 20% concentration of DO in the air for aeration decreased the lipid content of the microalgae [35]. This technique has been proven to increase the build-up of the lipid content in the *S. limacinum* cell [33].

3.2.2. Effect of carbon dioxide

To achieve the first stage of microalgal culture, the low CO_2 content of atmospheric air is sufficient for cell reproduction. Each microalgal strain has a different response to the CO_2 concentration. Thus, the period to maintain the CO_2 concentration in a culture medium is dependent on the species. The second stage, however, requires additional CO_2 to increase the lipid content.

Table 3 shows the effect of the CO_2 concentration on the microalgal biomass. When microalgae is cultivated in the presence of 6–8% CO_2, the biomass yield is up to 0.376 g/l/d, whereas the presence of 9–10% CO_2 concentrations has resulted in lower biomass yield

Table 1
Comparison of microalgae productivity.

Microalgae species	Yield (g/l/d)		Culture conditions[c]	Reference[d]
	Biomass[a]	Lipid[b]		
Botryococcus braunni	0.16	0.029–0.064	A[e]	[250]
Chaetoceros calcitrans	N/A	0.02214	C	[15]
Chlorella emersonii	0.25	0.157	D	[21]
Chlorella emersonii	0.36	0.122	D[f]	[2]
Chlorella minutissima	0.16	0.0912	D[g]	[21]
Chlorella vulgaris	0.37	0.148	D	[21]
Chlorella vulgaris	0.24	0.14	D[f]	[2]
Chlorella protothecoides	2.4–7.3	1.24–4.16	F	[18]
Chlorella protothecoides	3.6–4.1	1.6–1.7	I	[20]
Chlorella protothecoides	1.93	0.27–1.06	G	[19]
Chlorella protothecoides	1.3	0.654	H	[51]
Chlorella protothecoides	0.93	0.56	N/A	[23]
Isochrysis galbana	N/A	0.0207	C	[15]
Nannochloropsis sp.	0.48	0.142	L	[14]
Nannochloropsis sp.	0.3	0.204	B	[251]
Nannochloropsis sp.	0.09	0.025	K	[252]
Neochloris oleoabundans	0.09	0.0261	K	[252]
Neochloris oleoabundans	0.055	0.0126	J	[253]
Schizochytrium limacinum	1.88	1.38	M	[3]
Schizochytrium limacinum	0.186–2.0	0.22–0.54	N	[254]
Scenedesmus obliquus	0.15	0.27	O	[255]

[a] Biomass yield based on dry weight in gram per liter per day.
[b] Lipid yield based on dry weight in gram per liter per day.
[c] Based on method listed in Table 2.
[d] References as showed in reference list.
[e] Double concentration of all nutrients in Method A.
[f] As in Method D except cultivated in 230L photobioreactor and lit up with 130 μmol/m^2 s of light source.
[g] As in Method D but using Guillards Marine media.
N/A, data not available.

at 0.15 g/l/d [36,37]. A 2% increase of CO_2 in the culture medium has been shown to be capable of increasing the microalgal productivity [14]. It has been shown that an aeration rate of 1 l/min with a 5% CO_2 supplementation under illumination of 25 μmol/m^2 s at the surface of the reactor resulted in the production of 63% lipid content of *C. emersonii* [21]. Widjaja et al. [38] showed that the CO_2 concentration plays an important role in increasing the lipid content, lipid composition and biomass yield. Based on their study,

Table 2
Culture conditions of experimented microalgae.

Method	Media compositions	Culture system	*T* (°C)	Irradiance[a]
A	Prate media	Cultivated in conical flask with 1% CO_2 concentration	25	100
B	*f* media with nitrogen depletion	Cultivated in 20 l Flat Alveor panel and aerated with 0.6 l/min air contain 3% of CO_2	25	115–230
C	Conway media	10 l Polycarbonated Naglene bottle	27	120
D	Low nitrogen media	Cultivated in 2 l Stirred tank bioreactor aerated with 1 l/min air contains 5% of CO_2.	25	113
F	Using basal medium	Cultivated in 5 l Bioreactor, aerated with 3 l/min of air	28	N/A
G	The glucose solution was batch fed and was grown in autotrophic batch cultures	Cultivated in 5 l Fermenter aerated with 0.5 l/min	28	213
H	Modified basal media	Cultivated in Erlenmeyer flask.	28	h
I	Using liquid basal media supplemented with glucose: 30 g/l and yeast extract: 4 g/l, reducing sugar from JA: 30 g/l and yeast extract: 4 g/l.	Heterotrophically cultivated in 1 l Erlenmeyer flask	28	N/A
J	Using modified bold basal media with different nitrate concentration equal to 1.45 is most suitable	Cultivated in 1 l, 0.03 m depth airlift photobioreactor and aerated with 0.5 l/min of air	25	270
K	N/A	Cultivated in airlift bioreactor	N/A	150
L	Using f/2 media in artificial sea water	Cultivated in cylindrical glass photobioreactor and aerated with 2 l/min air contains 2% of CO_2[b]	26	300
M	ATCC 790 medium	Cultivated in 250 l Erlenmeyer flask	27	N/A
N	Basal medium, 1–5% increase of glucose improved the biomass production	Cultivated in 250 l Erlenmeyer flask	25	N/A
O	Using N11 media with KNO_3 was substitute with KCl for N depletion, $Na_2HPO_4{\cdot}H_2O$ and KH_2PO_4 were replace with equivalent concentration of Na_2SO_4 and KCl for P depletion	Cultivated in 150 l Erlenmeyer flask	25	75

N, nitrogen; P, phosphorus; *T*, temperature (°C); h, fermentation; N/A, data not available; JA, Jerusalem artichoke.
[a] Irradiance in micromoles per square meter per second (μmol/m^2 s).
[b] Semicontinuous with 1 day culture replacements.

Table 3
Tolerable concentration of CO_2 and biomass yield by culture of microalgae.

Microalgae species[a]	CO_2 concentration (%)[b]	Biomass yield (g/l/d)[c]	Reference[d]
Botryococcus braunii	5 (Air enriched with CO_2)	4.96	[248]
	5.5 (flue)	0.077	[249]
	10 (pure)	0.026	[249]
Chlorella sp.	5 (Air enriched with CO_2)	2.51	[248]
	6–8 (flue)	0.323–0.38	[37]
	6–8 (pure)	0.318–0.376	[37]
	9–10 (pure)	0.15	[36]
Chlorococcum litorelle	10–20 (pure)	0.19	[36]
Dunaliella tertiolecta	5 (Air enriched with CO_2)	2.72	[248]
Scendesmus sp.	5.5 (flue)	0.203	[249]
	10 (pure)	0.217	[249]
Spirulina platensis	5 (Air enriched with CO_2)	3.18	[248]
Spirulina sp.	6 (Purified CO_2)	0.22	[257]
Synechocystis aquatilis	10 (Air enriched with CO_2)	0.15	[256]
Monoraphidium minutum	2 (pure)	0.480	[14]

[a] Blank row refer to the species as in previous row.
[b] Based on either pure, flue or air enriched with CO_2.
[c] Based on daily yield per liter of media culture.
[d] Reference as showed in reference list.

the most significant CO_2 concentration for the production of a high lipid content is between 0.33 and 3.33%. However, Chiu et al. [14] showed that microalgal culture aerated with 2–15% CO_2 resulted in a microalgal specific growth rate of up to 0.66 per day. To summarize these results, the tolerable aeration rate of CO_2 for the growth of *Chlorella* is 0.33–15%.

3.2.3. Effect of the light conditions

The light intensity has different effects on microalgal species, as some species require more or less light energy to conduct the photosynthesis process. A light intensity between 76 and 600 μmol/m^2 s can be applied to culture microalgae [21,39]. During the first stage, light as low as 76 μmol/m^2 s should be applied to the culture medium. In the second stage, the harvested cultures can be exposed to a higher light intensity of approximately 240 μmol/m^2 s under batch conditions for 15 days. This technique allows for the transition to the aplanospore stage to be stimulated and the build-up of certain biochemical content such as astaxanthin. The literature also shows that this range of light intensity did not decrease the cell density of the cultures [34].

The dark-light cycle of the cultivation process was shown to have an effect on the cell lipid content during the second stage. Most experimental work on freshwater and marine microalgae was conducted under dark-light ratios of 12:12, 14:10, 10:14 and 16:8 [25,40,41].

3.2.4. Effect of the nitrogen supply

A high nitrogen (N) concentration is important during the first stage of the cultivation process to support the reproduction of microalgal cells, but the N concentration should be depleted in the second stage to levels that only support the synthesis of enzymes and the critical cell formation. Thus, in the second stage, any present carbon would be converted into lipids rather than proteins, whereas protein is important for algal growth in the first stage.

The mechanism behind the effect of the depletion of the N source is believed to be associated with the decreases in the intracellular chlorophyll and chloroplast numbers, where a large amount of phospholipids and glycolipids lie within the chloroplast. The depletion of the N source affects the intracellular consumption of the nitrogen pool to support the synthesis of cell material for further cell division. Thus, culturing under depleted nitrogen levels also inhibits microalgal growth. Thus, the concentration and duration of N that should be applied to microalgal culture is an important parameter to optimize the biomass and lipid productivity of microalgae.

To support the first stage of the culture process, up to 10 g/l of a N source can be applied to the microalgal culture. This amount promotes cell growth and resulted in low lipid production. During the second stage, the N amount ranging from 0% to 1% can be applied; this amount resulted in low growth rates and gradual increases of the lipid content that were roughly two times higher compared to normal culture [38,47,48]. Up to 63% of the chlorella lipid content can be achieved by applying a N-depleted medium over a 14-day growth period [21]. The nitrogen shortage in the culture medium reduces the protein and carbohydrate content of algae [48,49], thus the cultivation under N-depleted conditions is only suitable if the final product is biodiesel. It is critical to analyze the depletion period and amount of the N source that should be applied during the culture period. Among the limits that must be explored to apply this condition is the critical period of the depletion time that is suitable to increase the lipid production but will not decrease the microalgal growth.

3.2.5. Effect of the nitrogen source

It is important to select the most productive N source for the first stage. Among the N sources that can be used in the first stage are include urea, ammonium, nitrate and yeast extract. These N sources have no significant effect when added at concentrations between 0.85 and 1.7 g/l [50–53]. However, the higher the N concentration, the higher the biomass yield that can be achieved, and urea was shown as the most effective N source. The highest specific growth rate that was achieved is 0.0479 per hour, with a biomass productivity of approximately 18.7 g/l [50] when cultivated in a medium with urea as the N source. An organic source such as corn steep liquor can also be used as the N source. It is reported that a 37% total fatty acid content can be achieved by using 2 g/l (2%) corn steep liquor in the medium [54]. The organic N source can be an alternative to reduce the capital cost of microalgal cultivation, as this N source can be obtained from other biomass. However, other components of the medium such as the carbon source for heterotrophic growth and trace materials can also be a limiting factor of microalgal growth. The culture method, such as semi-continuous, batch, and fed-batch culture, also influences the effect of the N source on the biomass productivity.

3.3. Effect of climate

It may not be feasible to cultivate microalgae by controlling the temperature. Thus, it is important to culture microalgal species that can easily adapt to the climate conditions. Some microalgal species

grew better in a tropical rainforest, dry, wet, snow or highland climate. For this reason, the cultivation temperature of microalgae that have potential as a good biofuel feedstock is discussed thoroughly.

Schizochytrium sp. are well-known as a good lipid and DHA producer, *Botryococcus braunii* is well-known as a good hydrogen producer, and *Haematococcus pluvialis* is well-known as a good lipid producer. *Aurantiochytrium* sp. and *Chlorella* sp. can be cultivated in the range of 16–20 °C. Although temperatures as low as 10 °C were also reported to culture *Schizochytrium* sp., these temperatures resulted in lower lipid production compared to culture at temperatures greater than 16 °C [43–45].

The species that can be cultivated under temperature between 20 and 25 °C include *Chlorella* sp., *Schizochytrium* sp., *Aurantiochytrium* sp., *H. pluvialis* and *B. braunii* [34,42,61]. The strain of *Chlorella* is capable of accumulating up to 63% lipids when cultivated at 25 °C [21]; however, beyond this temperature, the production of DHA, which is significantly related to lipid production, of *S. limacinum* decreased sharply.

Between 25 and 30 °C, the *Chlorella* species, which is well-known as a good lipid producer, was reported grew better at 25–28 °C [18,21,23]. At this temperature, the *Chlorella* biomass contained approximately 57.8% lipid content. The *Chlorella* strains that were reported to be suitable for cultivation under this temperature include *C. protothecoides*, *C. vulgaris*, *C. minutissima* and *C. emersonii* [18–22]. Other microalgal species that are tolerant of this temperature range include *S. limacinum*, *B. braunii*, *N. oleoabundans*, *Parietochloris incisa*, *Nannochloropsis oculata*, *H. pluvialis*, *Platymonas subcordiformis*, *Chlorococcum littorale*, *Dictyosphaerium pulchellum*, *Scenedesmus falcatus*, *Nitzschia* sp., *Thalassiosira* sp., *Tetraselmis* sp., *Stichococcus* sp., *Synechocystis* sp. and *Synechococcus* sp. [3,9,14,21,24,137].

High-temperature climates in the range of 30–50 °C are rare climate conditions that are unlikely to favor microalgal culture. Only a few microalgal species can be cultivated under these conditions, including *C. vulgaris*, *Chlorella* species from Taiwan, *Chlorella sorokiana* and *Chlorella* isolated from hot springs [2,262]. In addition to *Chlorella* species, the well-known lutein-rich microalga *Scenedesmus almeriensis* was reported to have the highest temperature tolerance, as high as 48 °C [81]. Thus, this algae species can be cultivated in regions that have a continuous hot climate.

3.4. Phototrophic culture

In phototrophic culture, microalgal cells depend on light to reproduce. The absorbed energy from light is stored as ATP and NADPH, which are then used in the Calvin cycle to produce glucose. However, insufficient light intensity and CO_2 supply are always an issue for phototrophic culture. Cultivation in open pond depends on sunlight for growth. Thus, the photosynthesis process is limited to the time where light is present. Indoor growth, however, requires artificial light, which is costly. In addition to this, an uneven distribution of light intensity to the microalgal culture affects the productivity. It is reported that the phototrophic culture of microalgae amasses less lipid compared to heterotrophic culture; this is most likely because of the limited acyl groups between the chloroplast lipids. Thus, regular phototrophic culture is not sufficient to support microalgal lipid and biomass productivity to feasibly overcome the production costs compared with the current commercial feedstock. Thus, appropriate additional culture techniques such as two-stage growth should be applied when culturing microalgae under phototrophic conditions.

It was reported that additional CO_2 could increase the lipid and biomass productivity of microalgae. Depending on the regular CO_2 that is present in the air will result in low production. To supply additional CO_2, deep knowledge in terms of design is important to avoid the release of excess CO_2 into the atmosphere. In addition, the produced O_2 during normal photosynthesis is significant enough to lower or inhibit the activity of reversible hydrogenase, which produces H. High H production is also desired in addition to lipids as a bioenergy source. Thus, phototrophic culture is infeasible to produce H.

3.5. Heterotrophic culture

Heterotrophic culture consumes other organisms or organic waste containing carbons as an energy source instead of CO_2 and is independent of the light source for reproduction, thus it enables the high production of lipid compared to phototrophic microalgal cultivation [18,54]. Despite this advantage, some microalgal species such as *Chlorella* can adopt both growth conditions. In addition, Chlorella cultivation can be manipulated to utilize CO_2, which is produced from an industry plant. Both growth conditions have their own advantages and limitations because of the feasibility of the carbon sources. Heterotrophic microalgal cultivation is not practical for biofuel production when the carbon source has to be purchased, as it will increase the production cost.

The cost of the carbon source is one of the most discussed issues in heterotrophic growth that could hinder the success of this technique. The lipid content and biomass yield depend on the carbon type and concentration in the culture medium. Some of the most discussed carbon sources include glycerol, glucose and sweet sorghum. It was reported that the tolerable concentration of glycerol is within the range of 0.7–10% [33]. Microalgal production of more than 30% of DHA and biomass production can be achieved by using glycerol concentrations of 3–12% [42]. The literature also shows that 1 or 2% glycerol resulted in a higher lipid content of microalgae compared to a 5% addition to the medium [54]. In the cited work, a 5 or 10% glycerol supplementation did not have a significant effect.

Glucose has been used widely as carbon source for microalgal cultivation. Wu et al. [55] showed that a glucose concentration of 0.5–8% as the carbon source resulted in a lipid content of up to 44.48% in microalgae. The most favorable glucose concentration for high lipid production is 2%. Lipid contents as high as 57.8% can be achieved when the microalgae are cultivated in a control medium with glucose not exceeding 2.4% [18]. However, glucose at concentration 1.5–8% can be applied to microalgal cultures.

Sweet sorghum is another carbon source that was reported to be suitable to culturing microalgae. A study showed that an increase in the concentration of the carbon source led to high lipid yield. Lipid contents as high as 73.4% were achieved when 50% sweet sorghum juice was added into the culture medium instead of pure glucose [3]. The lipid content of microalgae cultivated in the presence of the enzymatic hydrolyzates of sweet sorghum, which contains 10 g/l (0.1%) of reducing sugars, resulted in a lipid content of 52.5%. This is 35.7% higher compared to results from cultivation using glucose [26]. However, the effect of sweet sorghum on the microalgal lipid production is still not widely explored, therefore many works will still need to be considered before applying sweet sorghum as the carbon source for commercial purposes. Other carbon sources that were used to culture microalgae, specifically *Chlorella*, are corn powder hydrolyzate and Jerusalem artichokes [19,20].

The production cost of heterotrophic microalgae can be reduced by utilizing the co-product of a refinery plant. It has been proven that the addition of crude glycerol as a carbon source, which is co-product of a biodiesel refinery plant, into the culture medium is capable of increasing microalgal productivity [33,42,54]. Thus, the heterotrophic culture can be beneficial when using available waste as the carbon sources.

4. Culture systems

4.1. Introduction

Microalgae can be cultivated in either open systems or closed systems, and both systems have a significant effect on the production cost of biofuel. Cultivation in an open system is usually conducted in an open pond or open tank that is widely exposed to the environment. Open ponds can be further categorized as raceway, circular, inclined and unmixed ponds. Cultivation in a closed system can be conducted in photobioreactor, which can be further categorized into many types including tubular, vertical, flat-plate, annular, fermenter-type and internally illuminated photobioreactors. An example of an open and closed system is shown in Fig. 2. As shown, the closed system (a tubular photobioreactor) has a more complicated system compared to the open system (a raceway pond). It also shows that the closed system requires a degassing column, which is required to remove the O_2 that is produced during the photosynthesis process. The light energy of both systems can be obtained directly from sunlight if cultivation takes place outdoors or by using an artificial light source for indoor cultivation.

Cultivation in a closed system has less contamination with the surroundings and can be easily controlled. The selection of a culture system depends on the final product: for biofuel production, microalgae can be cultivated in either open or closed systems. Pharmaceutics production, however, requires less contamination and thus should be cultivated only in closed systems.

Initially, open pond cultivation received considerable attention for the commercial cultivation of microalgae for biofuel production because of its easier set-up compared with photobioreactors. Among companies that have reported products for algae cultivation in open ponds are Aurora Biofuel, GreenFuel Technologies, PetroAlgae and Seambiotic [30,32,56,57]. Several unofficial commercial algae cultivation efforts in open ponds can be found online on the Oilgae [58] website. Recently, cultivation in photobioreactors has attracted more attention, as it is easy to control and is promising for higher productivities compared with open systems. However, cultivation in closed systems is costlier compared with open ponds. The additional costs include the light illumination, the CO_2 feed, the cultivation medium feed and the circulator system. In return, microalgal productivity in a photobioreactor is higher and has less contamination [55].

Table 4 shows the comparative analysis of open and closed systems. The annual biomass productivity of a closed system is higher than an open system [251,268]. However, under greenhouse cultivation conditions, an open system could produce up to 21 g/m^2/d [266]. Regardless an open or closed system, the maximum theoretical yield of oil from microalgae is 354,000 l/ha/yr [264]. The overall energy requirement for an open system (450 GJ/yr) is lower than for a closed system (729 GJ/yr) [263]. Much of the energy required comes from the pumping requirement, with a difference of approximately 50 W/m^3 between the open and closed systems [66,263].

4.2. Open pond

The brief comparison of types of open ponds can be shown in Table 5. As shown, raceway ponds are the most applicable for both the pilot-study level and commercial scale because of their easy set-up [59]. In addition, the productivities of raceway ponds have been reported to be 14–50 g/m^2/d. The productivity of a raceway pond can be increased by improving the CO_2 mass transfer [60]. It is also reported that the astaxanthin content of microalgae can be increased by applying the so-called two-stage growth process where one step cultivates microalgae in a raceway open pond [61].

Circular ponds, however, are capable of achieving algal growth rates as high as 21 g/m^2/d [62]. With the addition of organic carbon, higher algal growth rates can be achieved. The logic of the organic addition is to support the respiration process in the dark or to support the algal cell growth at the bottom of the pond where there is less exposure to sunlight.

Inclined ponds are rarely selected for microalgal cultivation, most likely because of their difficult operation in comparison to other types of open ponds. However, the literature shows that this culture system is capable of achieving a microalgal growth rate of up to 31 g/m^2/d [63]. Unmixed open ponds are generally used to culture *Dunaliella salina*. This pond type has low productivities of less than 1 g/m^2/d [62]. Thus, unmixed open ponds should be avoided for large-scale cultivation. In addition, unmixed open ponds are unsuitable for the cultivation of most algal species [63].

Most studies have shown that open ponds do not require high maintenance or set-up costs. However, open ponds are susceptible to contaminates and other fast-growing heterotrophic organisms, thus restricting the commercial production of algae. In addition, inefficient stirring mechanisms in open cultivation systems and poor mass transfer rates result in low biomass productivity. The mass transfer can be improved by attaching a carbonation column to improve the CO_2 transfer to the liquid phase to at least 90% [60]. Open ponds also require large areas of land. Other limitations that would be essentially uncontrollable in a large-scale mass cultivation system include the intensity, temperature and duration of sunlight. An example of an open pond design and system can be found in Sanchez et al. [64].

4.3. Photobioreactor

The vertical tubular reactor is the most popular type of photobioreactor that has high surface to volume ratios, low shear forces, low cost, absence of wall growth, high efficiency of CO_2 use efficiency, and the ability to use sunlight. Tubular photobioreactors can be used individually or arranged in parallel for better CO_2 consumption [65]. Flat-plate photobioreactors required a lower power supply for mass transfer compared to tubular photobioreactors,; 53 W/m^3 is sufficient to avoid the excessive build-up of DO in a flat-plate photobioreactor compared to the 2000–3000 W/m^3 required in a tubular photobioreactor [66]. The fundamental principle in all of photobioreactor designs is to reduce the light path and thus increase the light available to each cell. However, the design of a photobioreactor is more complicated compared to an open pond.

One study recommended a suitable pipe diameter to culture microalgae of approximately 0.1 m, with a flow velocity of 0.3–0.5 m/s. A diameter greater than 0.1 m will require an unrealistic culture velocity, which could damage the microalgal cells [67], and a diameter less than 0.03 m resulted in lower productivity [68]. This was associated with the light distribution and the mixing gas transfer between O_2 and CO_2. To achieve a higher flow and volume, multiple pipes can be arranged with the same common headers. Gas liquid mass transfer is an important feature of photobioreactors, and the biggest challenge to design the photobioreactor for high biomass productivity. As stated in the literature, poor mixing mass transfer increases the risk of CO_2 stripping in the photobioreactor, which inhibits the growth of the microalgae [69]. This is because as the photosynthesis activity increases, the O_2 concentration increases. The produced O_2 is significant enough to inhibit the growth of the microalgae. However, this cycle can be predicted by using a mathematical equation [70]. Another important feature is to achieve the correct light regime that allows for high photosynthetic rates, which can be estimated by using a modeling equation [71]. The most favorable photosynthetic rate could be eight quanta for every one molecule of O_2 [72]. Other reports also suggest that the lumostatic operation of a bubble column photobioreactor can be used to maintain the uptake light supply [73]. In addition to the light supply, the design volume

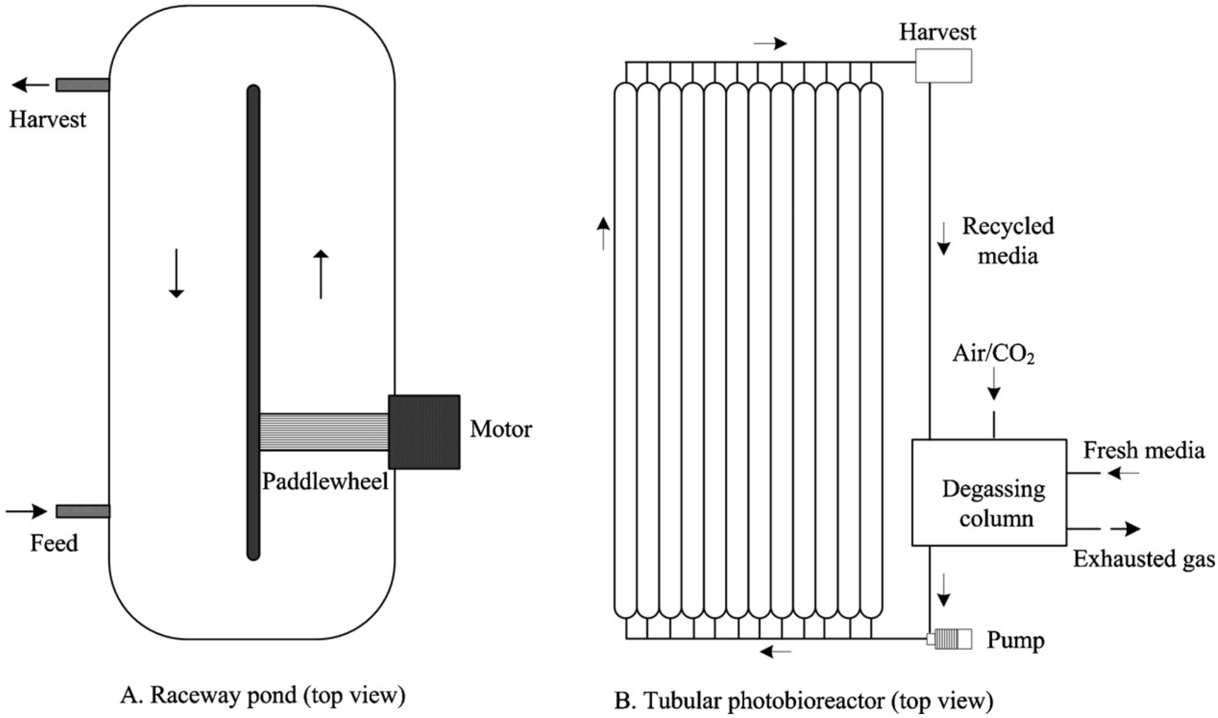


Fig. 2. An illustration of an example of an open and a closed system.

of a photobioreactor can be estimated based on the nutrient supply and oxygen removal from the photobioreactor system [74]. Some photobioreactors have been coupled with a heat-exchanger module to control the temperature of the culture medium.

Photobioreactors also have a higher efficiency when using flue gases such as CO_2 compared to open ponds [65], whereas a CO_2 removal efficiency of up to 82.3% can be achieved in an airlift bioreactor. This efficiency was measured based on the different inlet and outlet concentrations of CO_2 [13]. In addition to the high tolerance for CO_2 utilization, the airlift bioreactor was also reported to be suitable for batch, continuous and semi-continuous culture of microalgae [75].

4.4. Batch and continuous culture

Most laboratory work that has been reported in the literature was conducted in batch culture. Batch culture requires simpler design compared to continuous culture. Batch culture of microalgae is defined as a culture period where the cultivated microalgal cells are harvested at once. The batch operation usually remains the

Table 4
Comparative analysis of open and closed system.

Issue	Open system[a]	Closed system[b]	Reference[c]
Surface to volume ratio	Moderate	High	
Control of mass and gas transfer	Difficult	Easy	
Evaporation rate	High	Low	
Preheating	Low	High	
Irradiance supplied (MJ)	13.4	28.7	[265] [d]
Biomass productivity (t/ha/yr)	20	20–33	[251,268]
Volumetric productivity (kg/l/d)	0.035	0.27–0.56	[263]
Total energy consumption (GJ/yr)	450	729	[263]
Total energy content in 100 MT (GJ/yr)	3155.30	3155.30	[263]
Energy recovered as biomass (MJ)	1.2	2.7	[265] [e]
Energy produced as oil (GJ/yr)	1155.49	1155.49	[263] [f]
NER of oil production	2.56	1.58	[263] [g]
NER of biomass production	7.01	4.33	[263]
Cost estimation of TAG produced ($/kg)	7.5	33	[267] [h]
Cost estimation of FAME produced ($/kg)	4	25	[267]
Cost estimation of FFA produced ($/kg)	1	29	[267]

[a] Open system was based on race-way pond.
[b] The analysis of closed system was mainly based on tubular and flat-plate photobioreactor.
[c] References as indicated in reference list.
[d] Results was based on 50% photosynthetically active radiance (PAR).
[e] based on estimation that 1 g C contains 47.7 J.
[f] Energy produced as oil which was estimated based on 29.6% oil yield.
[g] Net energy ratio (NER) was estimated based on energy produced per energy requirements.
[h] The cost estimation is the highest gap between photobioreactor and open pond that ever reported in literature. The high cost form photobioreactor cultivation was estimated mostly from the processing equipment such as LED-lit photobioreactor with an LDPE dispensing tube, LED arrays, motorized stirrer and educator for nutrient supply. The price is in US Dollar.

Table 5
A brief comparison of open ponds properties, productivity and operation.

	Raceway	Circular	Inclined	Unmixed	References[a]
Operation	Operate with the aid of paddle wheels to make the water flow continuously	Operate with paddle wheels, usually applied for wastewater treatment	Operate based on gravity principle or with the aid of pump	Usually applied for Wastewater treatment	[258,259]
Properties	Good mixing and light distribution but poor in gas transfer, low hydrodynamic stress	Poor mixing, light distribution and gas transfer, low hydrodynamic stress	Poor mixing and gas transfer, very low hydrodynamic stress	Poor mixing and gas transfer, low hydrodynamic stress	[260]
Productivity	14–50 g/m^2/d, depends on location and weather conditions, was reported capable to produce about 30–32 ton/ha per annum	Up to 21 g/m^2/d, depends on location and weather conditions	31 g/m^2/d, depends on location and weather conditions	Less than 1 g/m^2/d, depends on location and weather conditions	[62,63,261]
Tested microalgae species	Suitable for most microalgae type include *Chlorella* sp., *Spirulina* sp., *Dunaliella* sp.	Suitable for most microalgae type include *Chlorella* sp., *Spirulina* sp., etc.	*Chlorella* sp., *Spirulina* sp., *Dunaliella* sp., *Haematococcus* sp., *Nannochloropsis* sp.	Not suitable for most microalgae. Suitable for *Spirulina* sp., *Dunaliella*	[63,258,259,261]

[a] based on reference number as indicated in reference list.

same even as the dilution factor is decreased and once harvested, the entire culture will be replaced with fresh microalgal cells and medium. The continuous culture, however, is conducted based on the dilution rate. When dilution reaches a certain point, half of the medium will be harvested and replaced with new medium to maintain the desire dilution, usually up to 0.67 per day. The microalgal biochemical contents such as lutein and exopolysaccharide (EPS) decrease as the dilution rate increases [80,81], and this dilution level is impossible to maintain in batch culture unless all or half of the medium is replaced with fresh medium. Cuaresma et al. [82] has also shows that biomass concentration and yield are depended on dilution rate of the culture medium.

The advantage for cultivation in batch culture is that a large number of measurements for microalgal analysis can be performed with a relatively simple setup [76]. Some literature shows that batch culture is capable of producing more biomass and biochemical compounds compared to continuous culture, for example, in the study conducted by Yongmanitchai and Ward [77], the biomass and EPA content for batch culture are 0.7 and 0.0825 g/l/d, respectively, whereas in continuous they are 0.51 and 0.0251 g/l/d, respectively. Under the same culture conditions for 5–9 day cell incubations, the batch culture favored *C. vulgaris* growth compared to continuous culture. Approximately 7×10^6 cells/bead was achieved in batch culture compared to 5×10^6 cells/bead under continuous growth [78]. However, in terms of the productivity, continuous culture has a higher biomass yield [79].

The effective use and design of continuous culture systems are capable of lowering the production cost by approximately 40% compared to the traditional batch culture. The economic analysis was performed based on the feed rate and daily harvest for continuous culture, whereas the batch culture was analyzed based on the mean yield of the harvested biomass [83]. The continuous culture system described by Sananurak et al. [84] can be applied for culturing microalgae. This system was originally used to culture microalgae for feeding to larva fish. However, this system can also be applied to the sole culture and harvest of microalgae.

The cultivation result shows that for most microalgal species, the effect of cultivation in batch or continuous culture on the productivity is small compared to the effect of the nutrient feed concentration such as the nitrogen and carbon ratios. The growth rate shows similar results in either batch or continuous systems [17]. For longer periods of cultivation, such as four months, higher microalgal production can be achieved in the continuous culture mode compared with batch culture [85]. The biomass and DHA productivity of microalgae cultivated under continuous culture is comparable to batch culture [86].

5. Harvesting techniques

5.1. Introduction

Harvesting microalgae can be difficult because of their small cell size. Therefore, harvesting can be costly, particularly those methods that involve a further downstream drying process. Thermal drying is more expensive than mechanical dewatering. According to Borodyanski and Konstantinov [87], approximately 25% of the production cost is incurred during the harvesting process, which includes the cost of electricity, reagents and the maintenance of the separation equipment. Microalgal harvesting usually involves flocculation followed by harvesting either by filtration, centrifugation, sedimentation or flotation; ultrasound techniques are still in development.

5.2. Flocculation of microalgal cells

Harvesting microalgae at the commercial scale usually involves a flocculant to reduce the time required to separate the medium from the algal cells. Flocculant agents are materials that have the ability to support the so-called bridging phenomena between two molecules, leading to the coagulation process [88,89]. Flocculation is a process of aggregating the microalgal cells to promote their separation, beginning with the addition of a material (a flocculant) into the medium, which disturbs the stability of the particles in suspension, including microscopic cells, causing them to aggregate [90]. Flocculants with higher molecular weights are generally more effective. High molecular weight flocculants can adsorb several particles at once, forming a three-dimensional matrix. When this occurs, the aggregated cells become easier to harvest. This is why the most effective flocculants are polymers, either natural or synthetic [91,92].

Flocculation is not a critical step in separating algal cells. However, the selection of inefficient or inappropriate flocculants can be costly. Organic flocculants can be obtained naturally or synthetically. Natural organic flocculants are based on natural polymers such as starch and mucilage, whereas synthetic organic flocculants are based on various monomers. The okra mucilage that was developed as a drag reducing agent could most likely be used as flocculant [93]. To reduce the drag in pipelines, the mucilage use the formation of the so-called bridge phenomena to ‘disturb’ the flow channel, and this ‘bridge phenomena’ could be used to flocculate microalgal cells in media.

Organic flocculants have an advantage over non-organic flocculants with regard to the dosage used to flocculate the particle. Chitosan, for instance, has been reported to be capable of

harvesting up to 98% of the microalgae, and the reported compatibility dosage varied from 0.2 to 0.4 g/l [94,95]. However, doses as low as 0.005 g/l were also reported to be suitable to culture freshwater algae [96], and doses as high as 2 g/l for saltwater resulted in a separation efficiency of more than 98% [97] The efficiency of chitosan as a flocculation agent was affected by the pH level, where pH ranges from 5 to 8 were suitable for the flocculation process. The modified cationic chitosan–polyacrylamide was reported to be efficient as a flocculant at pH 5.5 when the dosage was 0.08 g/l [98]. However, chitosan is expensive and its efficiency is reduced in saltwater.

Another natural cationic polymer that is commercially available is Greenfloc120, which is made from starch and was reported to be efficient as a flocculant to harvest freshwater microalgae [99]. In addition, this flocculant is also excellent for wastewater treatment [100]. According to You et al. [101], the combination of the cationic polymers chitosan and starch can perform much better than chitosan or starch alone. However, this work only tested the flocculation of the algae for wastewater treatment. Organic flocculants were reported to affect the efficiency of the filtration process. According to Ji et al. [102], the dosage of the organic flocculant had a significant effect on the mitigation of membrane fouling during the filtration process, and they suggested the optimal dosage of the chitosan flocculant for a sustainable filtration time was 1.5 g/l.

However, synthetic organic polyelectrolytes have been long known to be good flocculants to remove algae from water. Among the reported algae species that positively respond to this flocculant include *Thalassiosira pseudonana*, *Attheya septentrionalis*, *Nitzschia closterium*, *Tetraselmis suecica*, *Rhodomonas salina*, *C. calcitrans* (marine), *Skeletonema* sp. and *Chlorococcum* sp. Recent studies show that anionic, non-ionic and cationic polyelectrolytes are capable of separating up to 89.9% of microalgae from the culture medium [95,103]. Polymeric flocculants, however, are limited to the use with a marine microalgal medium with a salinity of less than 5 g/l [92]. To overcome this limit, polymeric flocculants are always used in conjunction with minute quantities of inorganic flocculants such as alum or lime. High concentrations of inorganics, specifically metal salts, are not suitable for agriculture purposes [104]. The tolerable dosage varies from 0.25 to 10 g/l, with separation efficiencies of up to 90% [95,103,105].

Inorganic flocculants are another type of flocculant that are made from the combinations of salts and metals such as ferric chloride or alum. Separation efficiencies of up to 90% were reported when using ferric chloride as a flocculant [105]. However, studies have shown that pure inorganic flocculants have disadvantages, such as the inability to coagulate fine particles and are not economically feasible when large amounts are required to separate the solid and liquid phases of a biomass-medium mixture, but inorganic flocculants are useful in improving the performance of polymeric flocculants in saltwater. Bioflocculants such as *Paenibacillus* species AM49 have been reported to achieve separation efficiencies as high as 95% [106] and are environmentally friendly and thus safe for agricultural products such as algal biomass. However, this flocculant requires a long time to flocculate microalgal cells.

Flocculants can be applied in many ways: in autoflocculation, as a combination of two or more different types of flocculants and with the aid of physical process such as air sparging. Algae harvested with a combination of polymer and salt show approximately 10–40% higher harvesting efficiencies compared to harvesting with polymer alone. Harvesting microalgae with the aid of air sparging at 2 l/min for approximately 2 min and pH of 0.7–5 also improved the flocculation efficiency [107,108].

Another technique that was discussed and is still under development is the so-called microbial flocculation. This technique adds a minute quantity of a microbial culture, as low as 1 g/l, into the microalgal culture to be separated. The microbes selected as the flocculating agent must able to release extracellular polymeric substances when depleted of nutrients. The microbe was feed with an organic substrate such as crude glycerol, making this microbe less expensive compared to other flocculating agents. In addition, this technique will not damage the microalgal cell, thus allowing culture medium to be reused without further treatment. It is reported that the recovery efficiency (RE), which is the ratio of mass of cells recovered to the total mass of cells, is more than 90% and the energy needed is equivalent to 0.893 kWh for every 103 kg of dry mass flocculated [109,110].

A flocculation technique called electrolytic flocculation that involves no flocculants and only requires electricity as low as 0.3 kWh/m^3 was also reported in the literature [111]. This technique was typically applied to remove the taxonomic group of algae in a reservoir for drinking water and has a removal efficiency as high as 90%.

After the flocculation process, the separated algal cells then continue to filtration, centrifugation, floatation or sedimentation before a further drying process. Combining filtration, centrifugation, floatation and sedimentation can also be applied in harvesting microalgae. Fig. 3 shows an example of a combination technique for harvesting microalgae. This process is based on harvesting by a combined flotation and filtration technique. In Fig. 3, the biomass is first treated with a flocculant and an acid in a mixer reactor before being pumped into a flotation column. Here, the flocculated algal biomass is transferred into a filtration unit by an overflow system, and the clarified medium is recycled to the culture units, either an open pond or a photobioreactor. The harvested biomass is pumped into a drying chamber before being advanced to the extraction unit where the algal oil is removed.

5.3. Centrifugation and direct filtration processes

Centrifugation is the most preferred method to harvest microalgae for laboratory study. This is because this technique does not required additional chemicals; however, this method requires more electrical energy compared to flocculation. In large-scale harvesting processes, centrifugation provided good recovery and thickened the slurry, but the currently available equipment for centrifugation processes is too expensive. This hinders the application of this technique for commercial purposes. Concentrating the biomass could improve the centrifugation efficiency [112]. However, to concentrate microalgae to 30% of dry particles requires approximately 1 MJ/kg of energy, which results in additional costs [113].

In contrast, the direct filtration process harvests microalgal biomass directly by using a microbial membrane which only allows algal cells to pass through. This technique appears to be the cheapest technique to harvest microalgae. However, this technique requires backwashing to maintain the efficiency of the membrane filter and is time-consuming.

5.4. Ultrasound as a method to harvest microalgae

Microalgal harvesting using an ultrasound technique is currently under development. This technique was first mentioned in Bosma et al. [114]. In this method, the microalgal cells experience a force that drives them into the planes of pressure nodes when they are exposed to an ultrasonic standing wave. When the field is switched off, the aggregated cells settle rapidly because of the gravitational forces. This technique requires further study before it can be applied on a large scale, especially in open ponds where contamination is high because this technique can not only coagulate microalgal cells but also other sediments such as mercury [115].

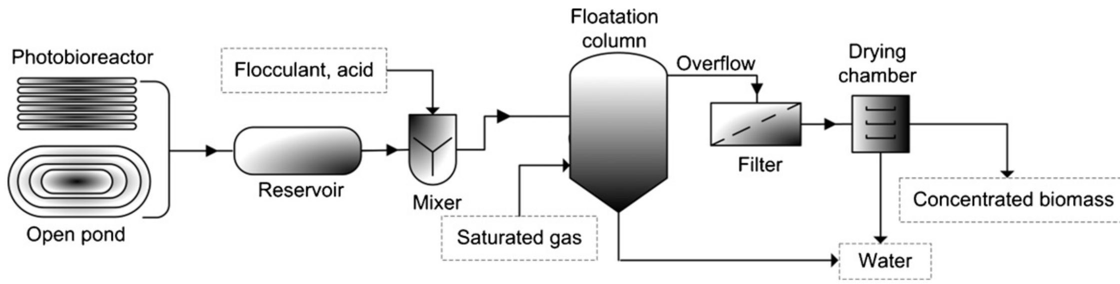


Fig. 3. The harvesting process of microalgae based on the flocculation and filtration technique.

5.5. Positively charged surface material as a microalgal harvester

The use of positively charged surface material as a "magnet" to attract and aggregate microalgal cells for harvesting purposes has not been mentioned previously in the literature. For this reason, the general idea is presented here with specifics on how the positively charged surface material could have a potential as a microalgal harvester. This idea is based on how differently charged materials are attracted to each other. Microalgal cells are naturally negatively charged. To verify the idea, small rod-shaped positively charged material could be tested in a beaker filled with the culture medium with 80% cell concentration. The harvesting efficiency can be studied based on the harvesting period and the cell concentration of microalgae that remain in the culture medium. On the pilot scale, the positively charged surface material can be setup inside the reservoir tank filled with a high concentration of microalgal biomass. The inlet microalgal biomass the from culture tank is fed at the bottom of the reservoir tank. The harvester is coated with the positively charged material (moving conveyer at 40°, thickness: 0.25 cm, width: 20 cm, length: 40 cm). The microalgal biomass that attaches to the charged-material can be removed as the conveyer passes the static scrubber. The scrubbed microalgal biomass can be accumulated into the storage tank by gravitational force. The overflow medium can be recycled to the culture tank. Based on the basis concept that positive and negative charges are attracted to each other, the negatively charged microalgal cells are expected to be attracted to the positively charged material. However, the strength of the charge and the type of material that will be used is expected to have a great impact on the harvesting efficiency and either decrease or increase the efficiency, and the biggest challenge for this idea is to find a suitable material and strengthen its potential. This idea differs from the electrolytic flocculation technique. Electrolytic flocculation causes the microalgae to lose their charge and forces them to form an aggregate at the surface. The use of a positively charged surface does not cause microalgae to lose their charge but forces them to attach and aggregate at the surface of a charged material, and the microalgae can be removed from the solution as the charged material moves out from the solution. However, this idea is just a general proposal which has not yet been tested in the laboratory.

6. Extraction of oil from microalgal biomass

6.1. Introduction

Microalgal oil can be extracted chemically or mechanically, similarly to other oleaginous biomass. Usually physical extraction requires an additional chemical as a solvent to enhance the extraction process. The most popular solvents include hexane and alcohol. The supercritical fluid extraction technique was also mentioned in the literature as having the highest extraction efficiency compared to other techniques. Physical techniques include expeller presses, electromechanical methods, ultrasonic extraction and soxhlet extractors. The purpose of the extraction process is to obtain oil from the algal cells to ease their conversion into biofuel or other agricultural products through biochemical or thermochemical means. It was reported that the extracted oil from microalgae contains more than 21.1 MJ/kg of heating value [116].

6.2. Conventional extraction processes

Conventional extraction techniques usually involve a dewatering process before the extraction process. Although it has been reported that algal biomass with a moisture level as high as 78.4% can be converted directly into fuel oil by thermochemical liquefaction from 300 to 360 °C and 10 MPa, the oil yield is much lower (25–44.8%) compared to a process with dewatering before extraction [117,118].

The dewatering process is conducted in an expeller or by pressing. Steaming or heating under high pressure before a mechanical pressing to extract the oil from the crops is the current method used commercially to extract oil from crops such as palm oil and jatropha. This technique can be applied to heat microalgae, however, the expeller or pressing process would be difficult to apply because of the micron-size of microalgae. This technique requires no chemical solvents, thus preserving the quality of the extracted oil. This simple technique extracts 70–75% of the oil from microalgal biomass. Many commercial manufacturers use combinations of chemical and mechanical methods to extract oil from vegetable crops.

Supercritical-fluid extraction is another process that has received increased attention as an important alternative to the traditional separation methods, with CO_2 being the most efficient solvent [119]. Carbon dioxide extraction is preferable because the compounds can be obtained without contamination by toxic organic solvents and without thermal degradation. Oil extracted by this technique is high-quality. However, this technique is time-consuming and is thus less efficient for commercial or large-scale production.

The OriginOil Company introduced and launched a new extraction method that does not require an upstream dewatering process and is capable of extracting oil from microalgae at a rate of five gallons for every minute, equivalent to 94–97% efficiency. This technique is the most efficient commercial technique thus far, and the cost can be reduced by omitting the dewatering process. The extraction involves breaking the cell walls by applying a combination of an electromagnetic field and a pH adjustment followed by tank settling or gravity clarification to separate the oil, water and biomass. However, a patent application for this technique is still pending [120,121].

6.3. Ultrasonic techniques to extract biomass oil

The ultrasonic technique is capable of increasing the microalgal oil yield by 50–500% compared to conventional methods. This yield is affected by the ultrasonic strength and solvent type. The

extraction times can be reduced up to 10-fold [122,123]. The ultrasonic strength and solvent type affect the efficiency of this technique. In addition to pure oil, other biochemical compounds in microalgal biomass such as carotenoids and chlorophyll can also be extracted by using ultrasound technique with comparable results to the supercritical method [124]. The microwave technique, which is similar to the ultrasonic technique, has also been reported to be capable of yielding higher unsaturated and essential fatty acids compared to typical extraction techniques such as water bath control. Approximately 76–77% of the total recoverable oil can be extracted within 30 min [125].

6.4. Solvent types to aid the extraction process

The solvents that are usually used for chemical extraction are from petroleum products, typically hexane and toluene. Hexane is suitable to extract oil from either dried microalgal biomass or microalgal paste [126]. However, applying a hexane solvent-extraction method requires further processing such as distillation to remove the excess or residual solvent from the product. Using multiple solvents was reported to be better than using only one [123,127].

A switchable solvent to extract lipids from dried biomass has been recently reported in the literature. This solvent can be removed from the extracted oil by using carbonated water and is mostly made from amides and a moderately hydrophilic solvent [128,129]. This solvent must have the ability to be hydrophobic when in air and hydrophilic when under an atmosphere of CO_2, as is the case for a tertiary amine [130,131].

7. Conversion processes of microalgae to biofuel

7.1. Introduction

The conversion of microalgae to biofuel can be classified as either a biochemical and thermochemical conversion process. The biochemical conversion processes of biofuel are transesterification and fermentation, which produce biodiesel and ethanol as the main products, respectively. The thermochemical processes can be categorized as pyrolysis, liquefaction, gasification and hydrogenation. The pyrolysis and liquefaction processes produces bio oil fuel as the main product, whereas gasification produces syngas and hydrogenation is a process to improve the biofuel or feedstock properties. The main issue for the conversion process is the downstream management of tar formation, particularly during the gasification and pyrolysis processes. Tar can result in the formation of tar aerosols and presents a high possibility of polymerizing into more complex structures [132]. Because this is unfavorable for hydrogen production, a gasification process necessitates carefully controlled temperature and pressure conditions and a suitable catalyst to produce syngas. Therefore, the downstream processes, specifically tar formation, are discussed thoroughly in this work.

7.2. Biodiesel production from microalgae

Biodiesel is one of the most well-known biofuel products from microalgae. Biodiesel productions from microalgae have been discussed thoroughly in the literature [133]. Biodiesel is produced by transesterification with glycerol as a side product. The heating value of biodiesel produced from microalgae is reported to be 41 MJ/kg [19] and complies with the US standard for biodiesel, ASTM 6571 [27].

Based on early reports, microalgae accumulates up to 73.4% lipid content, with the major lipid component as triglyceride [3,42]; therefore, the lipid content can be converted to biodiesel through a

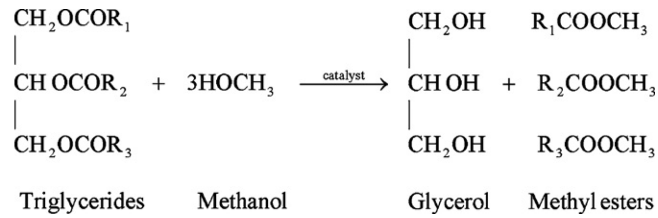


Fig. 4. The chemical reaction of the transesterification process.

transesterification process. Fig. 4 shows the general chemical reaction of the methyl ester production. Studies show that the enzyme from lipase is excellent for various vegetable oil conversions to methyl ester. Yields of more than 90% of crude oil can be achieved with conversion conditions of 35–50 °C at atmospheric pressure, where the molar ratio of oil to alcohol is 3:1–6:1 [134,135]. The estimation cost of the biodiesel produced from microalgae ranges from $3.69–4.20 per gallon [136].

The transesterification process occurs in a reactor where the blended methanol and catalyst react with the triglyceride present in the algal oil. The upstream product is then pumped to a separator tank. When using a base or acid catalyst, the upper layer is dominated by methyl ester, excess alcohol and the catalyst, whereas the lower level is dominated by glycerol [137,138]. This situation is similar to when an immobilized enzymatic catalyst is used except that the upper level only contains methyl ester. The methyl ester is then pumped into a washing column while the methyl ester resulting from the enzymatic catalysis process is forwarded directly into the drying unit.

Fig. 5 shows the main physical steps in methyl ester production based on an acidic and alkali catalyst, whereas Fig. 6 shows the general physical steps in methyl ester production based on an immobilized enzymatic catalyst. As shown in Fig. 5, the methanol and catalyst are blended before being pumped into a reactor tank, whereas this process is excluded when using an immobilized enzymatic catalyst. The immobilized enzymatic catalyst is designed to bond with the reactor as shown in the schematic diagram in Fig. 6. The dosage of the methanol and the catalyst are controlled to avoid excess amounts, which reduces the quality of main product and increases the energy required to remove the excess alcohol. The excess alcohol would be a large problem if an enzymatic catalyst used. Excess alcohol inhibits the enzyme activity and thus decreases the catalytic activity [139]. Overall, the transesterification of microalgae to produce biodiesel can be summarized as in Table 6.

The enzymatic transesterification process is influenced by the pH of the enzyme itself, the substrates concentration, and the spacing between the enzyme molecules and the substrate. The enzymatic catalyst does not change during the process, and it is effective to reuse it, which could reduce the cost of the process. However, if the enzyme mixes with the product or the solvent, it will require more downstream processing to separate them. In addition, free alcohols such as excess methanol and the produced glycerol, which is insoluble with the crude oil, promote dehydrogenases during the process and thus inhibit the catalytic activity. The methanol to oil ratio can be determined by conducting a laboratory-scale experiment before advancing into larger scales to avoid a high excess of methanol.

To avoid direct contact with free glycerol, the enzyme catalyst must be immobilized. There are three ways to immobilize an enzyme catalyst: carrier binding, cross-linking and entrapping the enzyme. Each technique has its limits and advantages. The carrier-binding method is the oldest and most preferable method for transesterification because it is efficient and economically viable [140,141]. This technique depends on the nature of the enzyme and

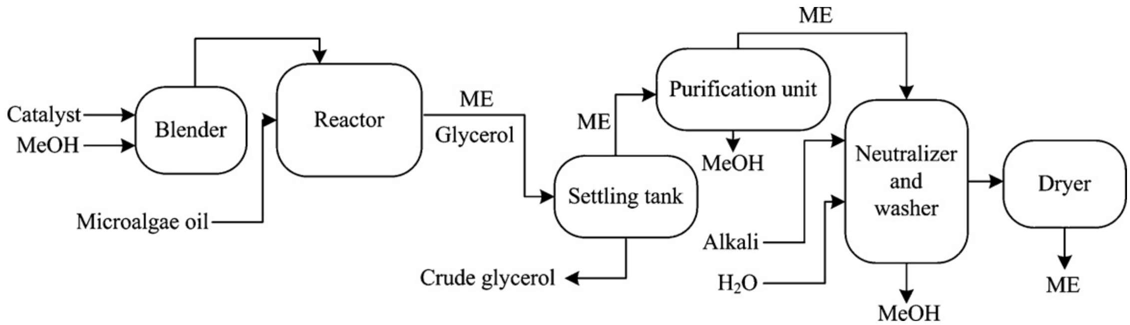


Fig. 5. An illustration of the physical steps in the transesterification process.

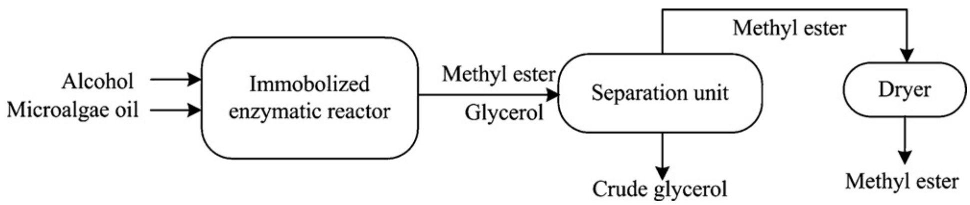


Fig. 6. An illustration of the immobilized transesterification process.

the substrate, including the particle size, surface area and molar ratio of hydrophilic to hydrophobic groups.

7.3. Ethanol production from microalgae

The ethanol trade represented approximately 10% of the world energy consumption in 2005, with Brazil being the main exporter [142]. Because ethanol is cheaper than gasoline, gasoline blended with less than 10% ethanol is usually required. The ethanol production from microalgae is primarily obtained through the fermentation of starch, sugar and cellulose contained in microalgal biomass [143]. It was reported that the carbohydrate content of microalgae is from 70 to 72% [144], where starch dominated the carbohydrate content and can be up to 60% by dry weight depending on the culture condition [145]. This starch content of microalgae can be improved by controlling the N or iron during cultivation [46,146].

Fig. 7 shows the summary of the upstream and downstream of microalgal processing steps to produce ethanol. The carbohydrates from the cell wall must be released before they can be used as a feedstock for fermentation; this can be accomplished by ultrasonic and explosive integration [147] or by hydrolytic enzymatic conversion of the biomass into a suitable fermentable feedstock [148]. One report also mentioned that the pretreatment of microalgae by dilute acid hydrolysis before enzymatic hydrolysis resulted in a higher glucose yield and up to 1.38% of fresh algal biomass [149]. This technique can likely be applied to treat the microalgal biomass. The pretreatment of microalgal biomass with a hydrolysis process by using an enzymatic catalyst resulted in more degraded fermentable material compared to acidic and alkaline catalysts [144]. The most effective enzyme concentration for a good ethanol yield was 0.001–0.05%, based on the volume unit of the enzyme for every weight unit of the feedstock [148].

There are four main reactions involved in ethanol fermentation. The first step is a glycolysis process, where one molecule of sugar, specifically glucose ($C_6H_{12}O_6$), is broken down into two pyruvate molecules ($CHCOCOO^-$). Glycolysis causes the reduction of the coenzymes: two molecules of adenosine diphosphate (ADP)

Table 6
Summary of transesterification process to produce biodiesel.

Parameter	Transesterification
Operation steps	1st: Feedstock blended with catalyst and alcohol 2nd: Transesterification process 3rd: Separation process 4th: Purification and neutralization 5th: Drying of final product
Feedstock type	Feedstock is crude microalgae oil composed mainly of TAG
Pretreatment of feedstock	Lipid oil is blended with catalyst and alcohol to improve the transesterification process. The ratio of feedstock to alcohol ranges from 3 to 6:1
Effect of immobilized enzymatic catalyst	Reduced the step of separation process between biodiesel and co-products. Immobilized enzymatic catalyst also increased the reaction rate
Effect of nonimmobilized catalyst on upstream product	Upstream product are biodiesel, excess alcohol and acid catalyst
Operation temperature	35–50 °C
Operation pressure	Atmospheric
Main product yield	Biodiesel which produced more than 90% of feedstock
Properties of main product	Energy content: 41 MJ/kg Density: 0.864 kg/l Viscosity at 40 °C: 5.2×10^{-4} Pa s
Estimation cost of main product	$3.69–4.20 per gallon
Co-products	Crude glycerol
Recyclable waste	Excess alcohol and catalyst: can be recycled to the system
Benefits of waste or co-products	Crude glycerol can be used as a feed into heterotrophic microalgae culture

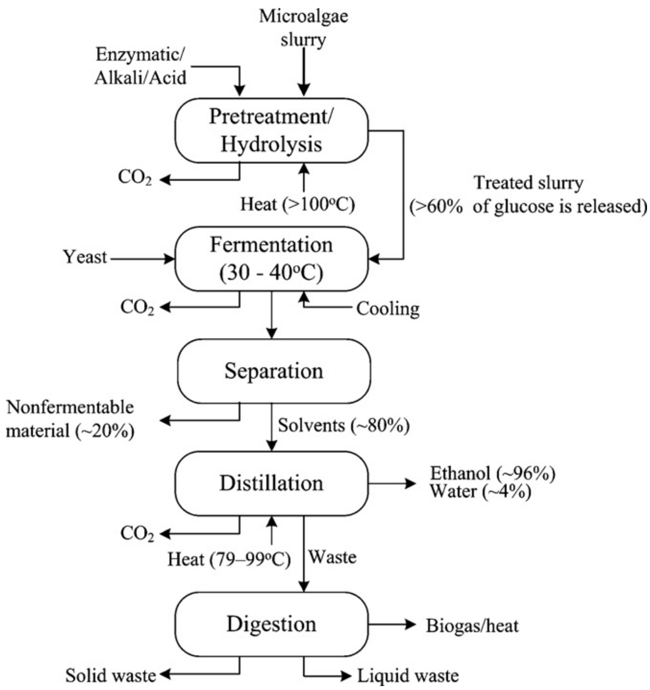


Fig. 7. An overall summary of ethanol production from microalgae.

are reduced to two molecules of ATP and two molecules of nicotinamide adenine dinucleotide (NAD^+) are reduced to two molecules of NADH. This process also produces water and hydrogen ions (H^+). The second step is the conversion of $CHCOCOO^-$ into acetaldehyde (CH_3CHO), catalyzed by pyruvate decarboxylase, which produces CO_2 and H^+. The third step is the conversion of the CH_3CHO produced in second step into ethanol ion ($C_2H_5O^-$) with the aid of the coenzyme NADH that was produced during the glycolysis process. Finally, the ethanol anion, which has similar properties to conventional ethanol, is protonated by hydrogen to produce ethanol (C_2H_5OH). During the fermentation process, CO_2 is produced as a side product.

Compared to untreated microalgae, pretreatment increased the efficiency of the fermentation process by more than 33% [150] and the ethanol production by more than 60% [151]. This estimation was based on the ethanol yield of treated microalgal biomass (51.2 g) and untreated microalgal biomass (20.1 g) for every 1 g of consumed glucose.

The pretreatment of microalgal biomass can be carried out with the aid of sulfuric acid or acetic acid. More than 50% of the glucose in a microalgal biomass slurry can be released by using sulfuric acid during the pretreatment process [151]. Ethanol yields of up to 0.26 g of ethanol per 1 g of microalgal biomass can be achieved [152]. However, pretreatment increases the energy consumption by up to 30% of the overall energy requirement for the fermentation process [147]. Ethanol yields from microalgae can be further improved by combining them with a hydrolysis separation process. Using a combined hydrolysis and fermentation method, approximately 0.235 g of ethanol can be produced from 1 g of microalgal biomass [54].

The ethanol production of microalgae can be improved by using yeast and an immobilized fermenter. The most preferable yeast for ethanol production is *Saccharomyces cerevisiae*, which has yields as high as 70 g/l [153,154]. Engineered yeast can also produce up to 61.8 g of ethanol from 1 l of cornstarch over a 72 h fermentation process [155]. During the fermentation process, the pH is maintained in the range of 6–9. A pH that is below 6 or over 9 could slow down the ethanol formation because of an excess of alkali. The immobilized reactor is capable of increasing ethanol yields by approximately five-fold relative to the 50% increases of glucose in feedstock [156].

The capital cost of ethanol production from algae was estimated to be approximately $1.75 per gallon by the Solution Recovery Service Company [157]. Thus, the estimated sale price of ethanol should be higher than this. The net life cycle energy consumption for the production of ethanol from microalgae was estimated to be 0.2–0.55 MJ for every 1 MJ of ethanol produced [158]. An overall summary of ethanol production from microalgae is shown in Table 7.

7.4. *Conversion of microalgal biomass to bio oil fuel*

7.4.1. *Perspective of the microalgal pyrolysis process*

The pyrolysis process of microalgal biomass is an anaerobic heating process that does not involve oxidation and occurs at high temperatures between 200 and 750 °C. Pyrolysis can be classified into two main categories: fast or slow pyrolysis, as shown in Fig. 8. The fast pyrolysis of biomass resulted in the production of bio oil (19–57.9% of the final product) and bio char [22,159]. The slow pyrolysis of biomass, however, resulted in the production of pyrolysis gas and bio char. Methane and carbon dioxide were the major components of the resulting gaseous product, which increases as the temperature increases and could comprise up to 76% of the final product at 600 °C [160,161].

The literature has proven that microalgae is a feasible feedstock for biomass pyrolysis. A pyrolytic rate of up to 87% can be achieved at 300–600 °C [161]. In addition, the bio oil produced from microalgae is more stable than the bio oil produced from traditional crops such as wood, although it is not as stable as fossil fuel [162]. The produced bio oil is mainly composed of aliphatic hydrocarbons, aromatic hydrocarbons, phenols, long-chain fatty acids and nitrogenized compounds [163]. Even at temperature as low as 300 °C, oil yields of up to 40% can still be produced [161]. The heating value of bio oil produced from microalgal biomass ranges from 30.7 to 41 MJ/kg, with viscosity of approximately 0.061 Pa s [22,163]. Higher oil yields with less oxygenic compound can be achieved in catalytic pyrolysis [164]. The amount of oxygenic compounds in catalytic bio oil is 19.5% compared to 30.1% in bio oil obtained by direct pyrolysis [165]. The catalyst that is used to aid the process can be recycled to the pyrolysis reactor. An energy recovery of bio oil of approximately 40% can be achieved under catalytic pyrolysis using $NaCO_3$ [166].

During the pyrolysis process, approximately 10–25% of the biomass is converted into char (solid porous carbon particles) and 10–30% is converted into a noncondensable gas [167]. The produced bio char is formed from the reactive pyrolysis vapor [168]. It was reported that the remaining bio char is one-third of the microalgal biomass [159]. The bio char products of fast pyrolysis have a higher heating value than the bio char products of slow pyrolysis [169]. The overall pyrolysis gas produced was 13–25% of the original biomass, had a heating value of 1.2–4.8 MJ/kg and was mainly composed of 9–17.5% CO_2 followed by 1–1.9% CH_4 [159].

Fig. 9 shows one of the commercial fast pyrolysis technologies that are currently applied by the Biomass Technology Group BV Company [170]. The process is based on the intensive mixing of biomass particles with hot sand particles as the catalyst in a modified rotating cone reactor. In Fig. 9, the pyrolysis process takes place in a reactor, resulting in vapor production and downstream products consisting of sand (sand is used as the catalyst) and char. Sand and char are recycled to the rotating cone combustor. The vapor is sent to a condenser, where it is rapidly cooled to yield the oil product and some gases. The rotating cone reactor, also known as the char combustor, is used to prepare the catalyst, such as the sand, before feeding it to the pyrolysis reactor. The pyrolysis product of

Table 7
Summary of fermentation process to produce ethanol.

Parameter	Fermentation
Operation steps	1st: Pretreatment to release carbohydrate 2nd: Fermentation 3rd: Separation 4th: Purification of ethanol
Feedstock conditions	Microalgae rich in carbohydrate composition. Sugar, starch and cellulose must be released to ease the fermentation process
Pretreatment of feedstock	Pretreatment will improve the ethanol yield more than 33% but increases energy requirement about 30%
Effect of enzymatic catalyst for pretreatment	Enzymatic catalyst for hydrolysis during pretreatment resulted in higher fermentable material
Preferable yeast	*Saccharomyces cerevisiae*
Operation temperature	30–40 °C
Operation pressure	Atmospheric
Main product yield	More than 23% of algal biomass
Properties of main product	Energy content: 29.7 MJ/kg Density: 0.772 kg/l Viscosity at 40 °C: 8.34×10^{-4} Pa s
Estimation cost of main product	More than $ 1.75 per gallon
Co-products	CO_2
Benefits of waste or co-products	The CO_2 produced can be feed into phototrophic microalgae culture, unfermentable cellulose can be further processed as animal feed supplement

microalgal biomass depends on the temperature and pressure conditions of the system. The optimum temperature for the thermal degradation of microalgal biomass is between 200 and 520 °C. The literature also shows that there is not much difference in the bio oil yield at 300 and 500 °C with a residence time of less than 20 min [160,161]. The pyrolysis process of microalgae can be summarized as shown in Table 8.

7.4.2. Perspective of the microalgal liquefaction process

The thermochemical liquefaction of biomass is a process that requires heating the biomass at high temperatures ranging from 200 to 500 °C with pressures greater than 20 bar in the presence of a catalyst. This process resulted in the production of bio oil yields ranging from 9 to 72% and gas mixture yields ranging from 6 to 20% [118,171,172]. This process also produced ash yields ranging from 0.2 to 0.5%. The product of the liquefaction process is also comparable with crude fuel, where the energy content of the bio oil ranges from 30 to 39 MJ/kg and the gaseous product also contain an energy content of more than 21 MJ/kg [169,173]. One advantage of liquefaction compared to other thermochemical process is its high tolerance of feedstock moisture content, which can be up to 65% [174,175]. Biomass feedstocks that have high moisture content

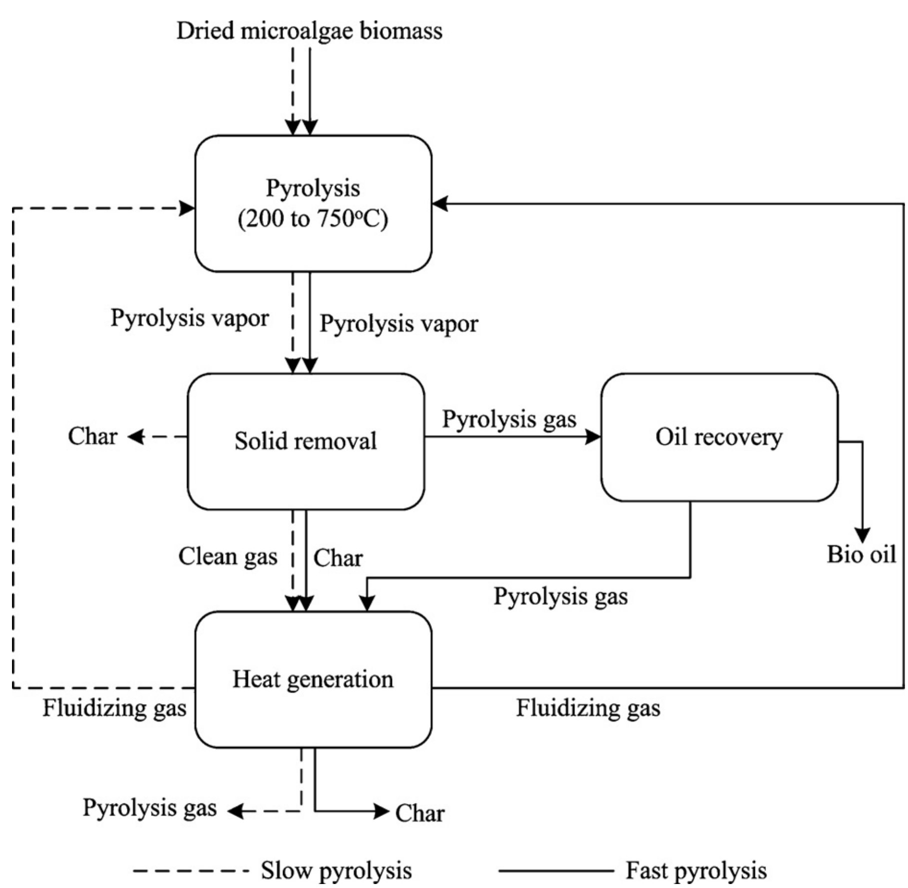


Fig. 8. The pyrolysis process for microalgae biomass.

Table 8
Summary of pyrolysis process to produce bio oil, pyrolysis gas and bio char.

Process	Pyrolysis
Operation steps	Fast pyrolysis 1st: Drying 2nd: Pyrolysis to produce pyrolysis vapor 3rd: Solid removal to separate char and pyrolysis gas 4th: Oil recovery to produce bio oil 5th: Heat generation to produce char Slow pyrolysis 1st: Drying 2nd: Pyrolysis to produce pyrolysis vapor 3rd: Solid removal to separate char and clean gas 4th: Heat generation to produce pyrolysis gas
Feedstock conditions	Biomass should be free from water content
Pretreatment of feedstock	Drying microalgae biomass
Operation temperature	300–750 °C
Operation pressure	Atmospheric
Main product yield	Bio oil: 28.6–57.9% (fast pyrolysis) Pyrolysis gas: 13–25% (slow pyrolysis)
Energy content of main product	Bio oil: 30.7–41 MJ/kg Pyrolysis gas: 1.2–4.8 MJ/kg
Properties of bio oil	Density: 0.92–0.98 kg/l Viscosity at 40 °C:0.02–0.061 Pa s
Co-products	Bio char: 10–25% (slow pyrolysis)
Recyclable waste	Fluidizing gas can be recycled to pyrolysis reactor
Benefits of waste or co-products	Some gaseous products are syngas which can be converted into methanol or hydrogenation feedstock. The bio char has many benefits specifically to agriculture field

with a ratio of solid to water of 1:10 resulted in a bio oil yield of approximately 36.9% [176].

The microalgal liquefaction process involves treating the biomass with a catalyst in a surge bin before sending it into a blender to make a biomass slurry [177]. The concentrated slurry of algal biomass is then blended with the hydrotreated recycled oil from the fractionation unit at temperatures ranging from 200 to 500 °C to make a biomass-oil slurry. The hot biomass-oil slurry from this blender is then pumped into a liquefaction reactor, where oxygen enters at the surface of the reactor and partial oxidation occurs. This process results in the production of synthesis gas, hydrogen and other hot gases and is shown in Fig. 10.

As shown in Fig. 10, the mixed hot gases are then pumped into a light-end stripper column to condense the product from the gas phase to the liquid phase. This process results in vapor production,

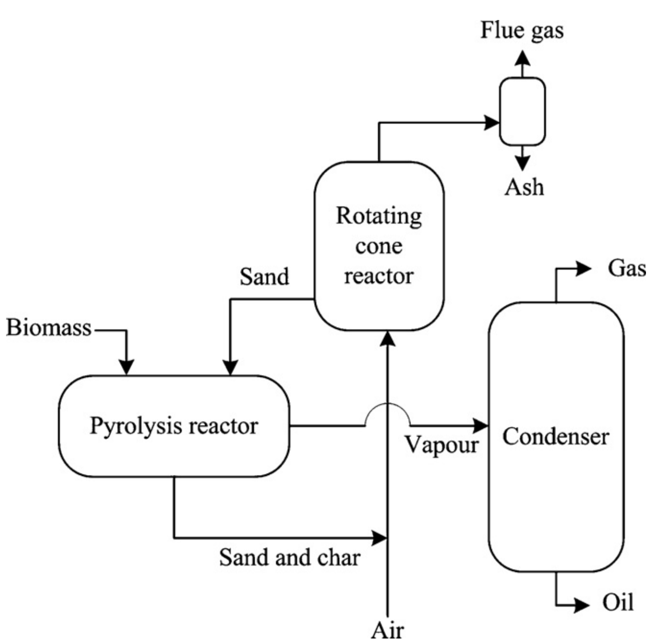


Fig. 9. The pyrolysis of biomass to produce bio oil fuel by using sand as a catalyst.

and the vapor is cooled and sent to decanter for further processing. The light oil is then sent to the oil filter to remove any solid particles while the crude oil is sent to the vacuum still system for further processing. The crude oil slurry is heated in a vacuum heater, resulting in the production of vapor and oil distillate. The vacuum still oil distillate, known as bio oil, is then sent to the oil filter and can be processed with the light oil from the end stripper column. The bio oil from the filtration unit can be further treated in catalytic hydrogenation before it is fed to a conventional fractionation unit. The oil is fractionated into lighter oil, which exits as the desired product, and the heavier oil is recycled to the biomass oil blender.

The liquefaction products of microalgae are mainly affected by the biomass composition and the liquefaction conditions of temperature, pressure, residence time and catalyst. The bio oil yield can be 5–25% higher than the lipid content of the microalgae depending on the other compounds in the oil such as carbohydrates [178]. *Dunaliella tertiolecta*, which is mainly composed of crude protein (63.6%) and fat (20.5%), produced a bio oil fuel yield of approximately 37% on an organic basis [174]. *Spirulina* sp., which is a well-known food supplement and rich in protein, was reported to produce a bio oil yield of up to 54% without a catalyst [179]. The *Microcystis viridis* strain, however, composed of 46% carbon, 7.3% hydrogen and 9.5% nitrogen, was reported to produce yields of up to 33 and 40% of bio oil and energy, respectively [180]. From these results, the microalgal liquefaction appears to have yields of bio oil fuel below 54%, lower than the other alternative thermochemical conversion processes such as pyrolysis and gasification. However, the oil yield can be improved by the addition of a catalyst.

The type of catalyst has a large effect on the gaseous product but is less sensitive to the crude oil of the liquefaction process. The highest methane yield is achieved when using Ru and Ni as a catalyst, whereas a zeolite catalyst is capable of producing a significant amount of N_2 [181]. Iron sulfide has been proven to be a feasible catalyst to produce higher oil yields and is soluble in n-hexane [182]. With iron sulfide as the catalyst, a yield of up to 66.9% was reported from *Spirulina* [179]. The optimal dosage of this catalyst was reported to be approximately 5–7% [183]. The literature shows that the aid of a catalyst increases the bio oil yield. However, the addition of a catalyst affects the quality of the produced bio oil. Without a catalyst, the produced bio oil tends to be a highly vis-

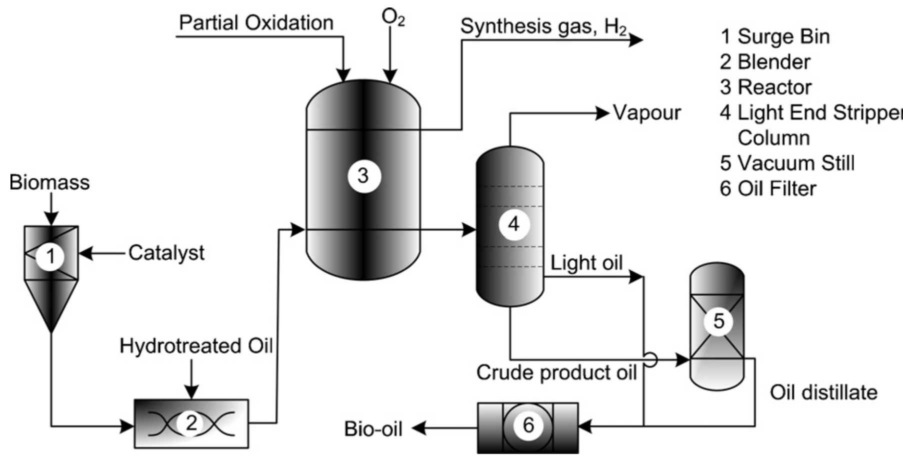


Fig. 10. The liquefaction process for microalgal biomass.

cous, dark-brown liquid with a foul odor [184]. The coliquefaction of microalgae with coal also helps to improve the conversion efficiency [117]. As liquefaction is the only thermochemical process that does not require a complex drying mechanism, this process is recommended for converting microalgal biomass to bio oil. The optimum reaction temperature for liquefaction suggested by Yang et al. [180] is 340 °C, with a residence time of 30 min and a catalyst dosage of 5%. Table 9 summarizes the liquefaction process of microalgal biomass.

7.5. The gasification of microalgae to produce syngas

Gasification of microalgae is a process in which the carbonaceous compounds of the biomass react with air, steam or oxygen at high temperature ranging from 200 to 700 °C in a gasifier and involves other thermochemical process such as pyrolysis and combustion. This resulted in production of clean H_2 [185] with yield ranging from 5 to 56% and CO with yield ranging from 9 to 52% [186–189]. Methane can be considered to be a co-product and is only produced in small amounts of approximately 2–25% [186,188]. However, the production of clean methane-rich gas can be achieved in catalyzed supercritical water gasification process where approximately 60–70% of the heating value from the microalgal biomass can be recovered as methane [190].

The hydrocarbon products of gasification can be further processed to produce methanol. At 1000 °C, the methanol production is approximately 64% (w/w) based on the biomass weight. The ratio of energy produced to energy required to produce the methanol from the gasification process is 1.1 [191]. The biomass gasification also produced unwanted products in small quantities such as water, ash and tar, which cause various problems with the main product yield. The tar produced can range from 0.1 to 20% depending on the gasifier agent and type of reactor, either an updraft or a downdraft reactor [192]. The syngas produced from the gasification process can be predicted by a correlation equation based on the hydrogen mole percentage and the temperature in the dry gas; this equation can be found in Cohce et al. [193].

Fig. 11 shows the thermochemical processes of microalgal biomass gasification that occurred in a general gasifier reactor to produce syngas and the possibility of using the hydrocarbon products as a synthesis feedstock to produce methanol. The drying process takes place at temperature ranges from 0 to 300 °C. As the temperature increases to 500 °C, the dried biomass is pyrolyzed into combustible gases such as CO_2, HC, vaporized tar or oil, residue MeOH and solid char. Partial oxidation or combustion processes occur when the steam or oxygen reacts with these gases at high temperatures of up to 700 °C. This process produced CO, H_2, methane, CO_2 and traces of hydrocarbon products. The produced H_2 is desirable for various purposes in chemical industries besides as a clean energy source. It is also used as a reactant in hydrogenation processes or to saturate compounds.

The gasification process is applicable for biomass with moisture contents of less than 15% [194]. However, moisture contents of up to 40% in microalgal biomass were reported to be tolerable for the gasification process [189]. An increasing moisture content degrades the gasifier performance and the energy content of the syngas produced. The high heating value (HHV) of the produced syngas at 5 and 30% moisture is 5.138 and 3.338 MJ/kg, respectively, while the cold gas efficiency (CGE) is 73.81 and 44.24%, respectively [195], showing that the moisture content of biomass has a

Table 9
Summary of liquefaction process to produce bio oil.

Parameter	Liquefaction
Operation steps	1st: Biomass blinding with catalyst 2nd: Biomass slurry mixed with hydrotreated oil 3rd: Liquefaction/partial oxidation 4th: Condensing 5th: Filtration
Feedstock conditions	Tolerable moisture content of biomass up to 65%
Pretreatment of feedstock	The feedstock of biomass is pre-treated with catalyst in surge bin to make microalgae slurry
Preferable catalyst	Ni based catalyst
Operation temperature	200–500 °C
Operation pressure	2–25 MPa
Main product yield	Bio oil
Energy content of main product	30–39 MJ/kg
Co-products	Gaseous product: methane (6–20%) with 21 MJ/kg heating value
Recyclable waste	Aqueous co product can be used as feed to microalgae culture

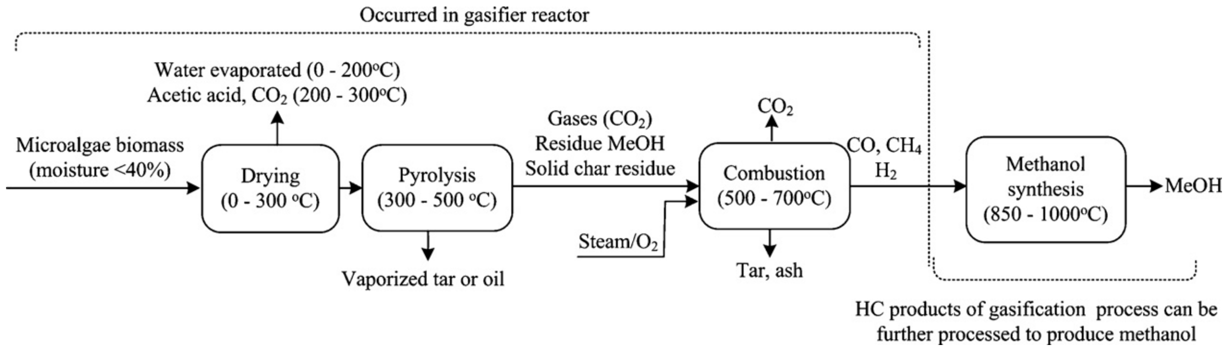


Fig. 11. The gasification process for microalgae biomass to produce syngas and methanol.

strong effect on the syngas produced. Thus, it is desired to improve the energy content of the produced syngas, which can be achieved by the pretreatment process. The biomass pretreatment through the torrefaction process improved the energy content of the syngas by 4%, where the energy content of the produced syngas was 72.6% compared to 68.6% for direct gasification [196]. The torrefaction process requires approximately 0.6–1 MJ/kg of endothermic energy. As a result, the torrefied biomass energy content is approximately 21–22 MJ/kg higher compared to untreated biomass [197]. By comparing the consumed energy with the produced energy, the pretreatment of the biomass before the gasification process provides an advantage to the biofuel production cost.

By increasing the gasification temperature and the catalyst concentration to aid the gasification process, a higher H_2 yield can be achieved. Among the catalysts that are usually used are dolomite, alkali catalysts such as nickel, and potassium carbonate [198]. The addition of a catalyst, specifically the nickel-based catalyst, reduced the tar production to as low as 5% at temperatures ranging from 500 to 900 °C [199]. The catalyst addition also increased the gasification efficiency of microalgal biomass, specifically *C. vulgaris*, up to 84% [188]. The gasification agent also affects the syngas yield. Using air as the gasification agent could produce a higher gas yield that is cleaner and richer in H_2 rather than steam [186,200]. However, more tar is produced with this method. This tar can be reduced by increasing the airflow rate during the gasification process.

The syngas produced is feasible if the energy content of the produced syngas is higher compared to the energy required during the gasification process. The energy content of the produced syngas was estimated to be 8000 kJ/N m^3 and the wasted energy that can be recovered is approximately 23% of the total energy input because of the heat loss [201]. The syngas production from biomass is summarized in Table 10.

7.6. The hydrogenation process to improve microalgal feedstock and biofuel properties

Hydrogenation of a carboneous feedstock is the process of adding or reacting H_2 into the double bonds of hydrocarbons. The factors that affect the hydrogenation efficiency include the temperature, reaction time, catalyst type and feedstock impurities [202]. This reaction results in the production of better feedstocks for other process or products that have lower molecular weights. For instance, the total saturated fatty acid of FAME increased from 29.3 to 76.2% after undergoing 2 h of the hydrogenation process [203]. Conversion rates of sugar to sugar alcohol were reported to be as high as 95% in the literature [204]. This shows that hydrogenation is indeed one of the ways to improve the product yield of an energy source.

The hydrogenation process can also be applied directly to convert biomass into bio oil. Chin and Engel [205] has proven that microalgal biomass, specifically *Chlorella pyrenoidosa*, is capable of achieving 50% oil yield in a batch autoclave with a hydrogen pressure of 0.98–147 bar. The product yield of the hydrogenation process can be further improved by combining it with the dehydration and aldol-condensation processes [206]. These combined processes yield approximately 14.8–20.3% of C_5 to C_{15} compounds [207].

The hydrogenation process of microalgal biomass requires three stages. The first stage is to fix the contact between the gaseous hydrogen and gaseous hydrocarbons. The second stage is to establish a liquid phase that is a mixture of solvent and liquid products, and the third stage is to establish contact between the algal biomass and the catalyst.

In the normal hydrogenation process of an unsaturated substrate, fat and mineral oil are usually reacted with hydrogen in a catalytic fixed-bed reactor. The biomass or unsaturated hydrocarbons are fed into a catalytic fixed bed, resulting in the production of steam. A flash separator is used to separate the hydrogenated feeds into two components: light gasses, such as untreated hydrogen, methane and propane, and a liquid fraction. The light gasses can be recycled into the fixed bed or purified for other purposes. The liquid fraction is then further separated through a fractionation column, resulting in the production of gasoline, kerosene and biodiesel. These processes are sketched in Fig. 12.

However, this opinion is based only on the current research found in the literature. As there have been few reports to-date on the hydrogenation of microalgal biomass, this will likely provide an interesting future research topic.

7.7. Downstream management based on by-products of the conversion processes

7.7.1. By-products of biodiesel productions

Downstream processes of biodiesel produced from the transesterification process depend on the type and method of catalyst. Three important aspects require specific attention: the purification of the main product, biodiesel, the by-products, glycerol, soap, excess alcohol and trace amounts of water, and the waste products. Effective downstream processing is important to recover energy that can be recouped from the entire process.

The produced methyl ester has a lower density than glycerol and thus can be separated easily by using a settling tank in large-scale production. For small-scale production, the methyl ester and glycerol produced from batch process can be separated by using membrane technology [208] in which a ceramic membrane having pore diameter 0.2 μm is used at 2 bar applied pressure. This resulted in a stable permeate flux of approximately 78.4 kg/m^2 h and a glycerol retention of 99.4% [209]. The separated methyl ester from glycerol is further purified in a distillation unit where a large amount of excess alcohol is removed, resulting in an approximately 99.6% purity of the methyl ester.

Table 10
Summary of gasification process to produce syngas.

Parameter	Gasification
Operation steps	1st: Pretreatment by torrefaction 2nd: Drying 3rd: Pyrolysis 4th: Combustion
Feedstock conditions	Moisture less than 40%, the biomass particle must carries less nitrogen and alkali component to reduce the impurities of products
Pretreatment of feedstock	Torrefaction process can be done on large biomass particle which improve about 4% of the syngas energy content. However, microalgae biomass does not need for this pretreatment
Preferable catalyst	Dolomite, nickel, potassium carbonate
Gasification agent	Air, pure steam, steam-O_2 mixture
Operation temperature	0–700 °C
Operation pressure	Atmospheric
Main product yield (v/v)	H_2: 5–56% CO: 9–52%
Co-products	CH_4 and other HC products, CO_2, tar: up to 20%, ash
Benefits of waste or co-products	The hydrocarbon can be synthesized for MeOH production (Yield: 64% of biomass)

The washed methyl ester is then sent to a neutralizer and washing column. The neutralized methyl ester then undergoes a drying process and is ready to be marketed. During the washing process, a low-velocity water spray is injected at the top of the column to remove the residual alcohol and catalyst. The residual alcohol and catalyst can be recycled to the reactor. The by-product glycerol, which is 10% of the overall products [210], is either purified and recycled into the system or sold as crude glycerol. Even without the purification process, the crude glycerol has high potential as a carbon source for microalgal cultivation [3].

7.7.2. By-products from bio oil fuel production

As in the gasification process, tar formation in slow pyrolysis and flash pyrolysis affects the quality of the bio oil produced and is again associated with the polymerization process of fine tar in the reactor. This tar can be removed by filtration. However, the alkaline material concentrated in the tar will dissolve in bio oil. Based on a study conducted by Devi et al. [211], the prevention or elimination of the tars inside the gasifier itself is the most efficient method of dealing with the tar formation. A Ni-based catalyst in the gasifier is one of the most effective catalysts that not only reduces the tar formation but also decreases the quantity of nitrogenous compounds [212].

Another issue is associated with the final product of the hydrogenation process. The content of the hydrogenated fatty acids in the biomass resulted in a high melting point and lowered the iodine value (IV) of the oil. Thus, the biodiesel produced from the hydrogenated oil is less likely to be oxidized and polymerized but will have a higher melting point than unhydrogenated oil.

This increases the risk of fuel filters becoming plugged in cold or even just cool weather, and thus this product is best used as a summer fuel. This is one likely reason that the hydrogenation process is less popular compared to transesterification for biodiesel production from microalgae. However, the issue of determining a catalyst that is suitable to be used for the reaction was the major concern of the transesterification process [213–216].

The aqueous co-product produced during the liquefaction process can be recycled to feed the microalgal culture. The ACP product was reported to be rich in nitrogen, phosphorus and potassium. The growth rate of microalgae cultured in medium that contains 0.1% aqueous co-product was reported to be 50% lower compared to microalgae cultured in an established medium such as BG11 [217].

The main downstream challenge of the gasification and pyrolysis process is the formation of tar, ash and char, which is filtered by a gas scrubber before the catalytic gas-shift reaction takes place. The tar can be cracked at 1000 °C, however, this requires a high heating energy [218]. Another way to reduce the formation of tar is to use additives such as dolomite, olivine, or char inside the gasifier [219].

In contrast, ash formation can be resolved by employing fractionation and leaching inside the reactor. However, this technique can also have an undesirable side effect, where fractionation can reduce the quality of the remaining ash, and leaching can remove the inorganic fraction of the biomass.

7.7.3. Waste products from ethanol production

The nonfermentable products and the solvent from the fermentation process can be separated by centrifugation or filtration. The solvent compositions include ethanol, residual catalyst and water. The nonfermentable or residual slurry consists of essential proteins, lipids and organic acids or alkalines. This residual slurry can be used as a feedstock for methane production, which can reduce the waste from 3.3 to 10% [147], or can be used to rupture the cell to release the proteins or enzymes as a by-product. The rupture of these cells is difficult because of the deliberation of deoxyribonucleic acid (DNA). This deliberated DNA increases the viscosity of the suspension, and a high viscosity of the suspension causes a problem with the pumping system [220].

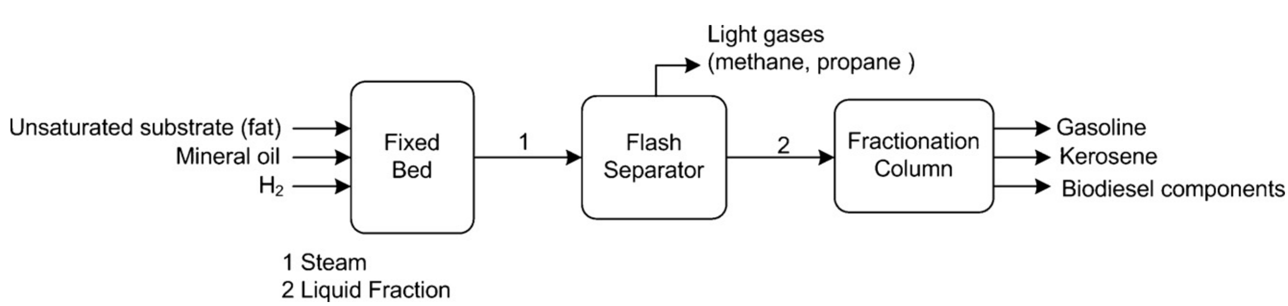


Fig. 12. The hydrogenation process to produce bio oil fuel.

The distillation process is the easiest way to purify ethanol. So far, the design of distillation equipment is only capable of purifying ethanol up to 95.6% in order to avoid the loss of the main product during the process. If the evaporated water used is recycled to the system, the overall energy consumption to produce ethanol is approximately 41.96 MJ/l [221]; this can be improved if a pervaporation method is used instead of a distillation process.

8. Other benefits of microalgae

8.1. Introduction

Microalgae are versatile organisms that can be incorporated into many fields. In addition to biofuels, this work also notes other benefits of microalgae. Among the discussed benefits are the potential of microalgae to be used as a CO_2 sequester, in wastewater treatment and as a pharmaceutics feedstock.

8.2. Potential as a carbon dioxide sequester

The microalgal growth rate is approximately 50 times higher than terrestrial plants [31]. Microalgae have higher photosynthetic efficiencies than terrestrial plants and are more efficient in capturing carbon [222]. The microalgal species that were tested to be suitable for CO_2 fixation include *Chlorella* species, *Spirulina platensis*, *Emiliania huxley*, *Phaeodactylum*, and *Nannochloropsis* sp. [223–225]. In addition to pure CO_2, flue gases from industrial plants were also reported to be capable of being used as a feed to phototrophic microalgae. The use of flue gas direct in the cultivation system does not adversely affect algal growth [223], which shows that microalgae are relevant to utilize the CO_2 emission from power plants. In addition, the analysis of microalgae shows a double benefit of converting CO_2 into energy, and this production of green energy has been shown as reliable and possible [226]. The use of microalgae as a carbon sequestrator is only considered feasible if they are used as biofuel feedstock rather than merely as a carbon sequester. There are specific parameters that must be studied before using microalgae for CO_2 sequestering purposes. These parameters include the analysis of certain systems with respect to how efficiently the microalgae could use CO_2 in order to avoid the release of excess CO_2 into the atmosphere. This parameter is important, as an open system may contain various species of microalgae that will have different utilization efficiencies. It is also important to design an effective closed or open system. Closed systems seem to allow for easy control of the CO_2 compared with open systems because open system cultivation directly releases excess CO_2 into the atmosphere.

8.3. Microalgae for wastewater treatment

Because the nature of microalgae requires a variety of nutrients, it is possible to incorporate microalgae in wastewater treatment. Wastewater has a significantly higher content of the individual amino acids that support the growth of microalgae. The literature has shown that microalgae are capable of reducing the chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in wastewater. Among microalgal species that were reported to be suitable to treat wastewater include *Scendesmus* sp., *Chlorella* sp., and *Chlamydomonas reinhardtii* [227–230]. The removal rate of metal ions such as aluminum, calcium, ferum, mangan and magnesium was also varied among the microalgae species, ranging from 50 to 99% [228,231]. However, a major challenge in achieving the double benefit of microalgae is determining a way that allows for downstream processing that is suitable for producing biofuel and other bioproducts [232].

8.4. Potential as a pharmaceutical feedstock

Microalgae have a high potential to provide pharmaceutical feedstocks such as amino acids, carotenoids, fatty acids, vitamins and antioxidants. The literature shows that microalgae, specifically *S. limacinum* and *Crypthecodinium cohnii*, are rich in the Omega-3 compounds docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which is important to prevent cardiovascular disease. A DHA content of up to 70% of the total oil yield of microalgae (33.9–50%) can be achieved by using supercritical CO_2 extraction [233,234]. The production of DHA from microalgae can be improved by manipulating the culture conditions. Using crude glycerol in the heterotrophic growth of microalgae resulted in the production of a higher DHA content compared to the other biochemical content. This increase in DHA will make the purification process easier [86,235]. Others microalgal species such as *Chlorella* sp. were found to be rich in the carotenoids canthaxanthin and astaxanthin [236,237]. Carotenoids are important to prevent the abnormal growth of cells and heart disease by blocking the formation of low-density lipoproteins (LDL cholesterol). In addition, *Dunaliella salina* produces natural β-carotene (a mixture of *cis* and *trans* variants) in high yields [238,239]. Beta-carotene is a precursor to vitamin A, which is essential for many functions in the human body such as vision, growth, and bone development and acts as a coenzyme and has roles in balancing effect of hormones. The *Spirulina* sp. are capable of producing large amounts of γ-linolenic acid. The lipid fraction of *Spirulina* sp. is composed of neutral lipids, glycolipids and phospholipids, which accounted for 77.0, 15.6 and 7.4%, respectively, of the total composition. From these amounts, the total lipid fraction of γ-linolenic acid is 94% of the total glycolipid fraction [240].

9. Cost benefit analysis

9.1. Introduction

In transforming microalgae to biofuel, one of the most important aspects that need to be deeply studied is the cultivation process. The cost and benefit of the culture approach that is selected for transforming microalgae to biofuel are discussed thoroughly. This includes the cost of the phototrophic and heterotrophic approaches for culturing microalgae. Among the parameters that will be considered are the cost of the inorganic carbon as the energy source for heterotrophic growth and the comparison of the final lipid and biomass yield with phototrophic growth. Thus, these options were analyzed here and compared by taking consideration the final yield.

9.2. Heterotrophic and phototrophic culture

The cultivation costs for microalgae depend on the cultivation technique and the nutrient sources. Carbon is the most important nutrient for microalgae because it affects the microalgal lipid contents. Previous studies have shown that using sweet sorghum as a carbon source for heterotrophic growth leads to higher lipid production than other carbon sources such as industrial flue gas or crude glycerol from a biodiesel refinery [3]. Because of this result, the feasibility of using sweet sorghum as the carbon source is discussed in this work.

To grow sweet sorghum, approximately 4000 m^3 of water is needed for a four-month period, after which it can be harvested, resulting in a grain yield of 2 ton/ha. According to William [241], the cultivation cost of sweet sorghum is $258 per ha. However, the cultivation cost of this crop can be as high as $857.6 per ha as estimated by Claassen et al. [242], which resulted in the production of fresh biomass from 50 to 140 ton/ha where the preharvest cost varied from $190 to $735.76 per ha [242,243]. Based on this number,

the price of sweet sorghum is estimated to be more than $477.88 per ton or $0.48 per kg of biomass.

However, Gnansounou et al. [244] estimated the price of sweet sorghum juice to be much lower than that given by Bennet and Anex [243] at $26.90 per ton or $0.027 per kg. In contrast, the National Agricultural Statistical Services [245] stated the average price of sweet sorghum in 2009 to be $5.90 per hundred weight, equivalent to $0.134 per kg. This shows that the cost of sweet sorghum has varied from $0.027 to $0.48 per kg and is mainly affected by the cultivation costs include the land, water, fertilizer and labor requirements. The feasibility study that was reported in the literature shows that the higher the biomass yield, the higher the cultivation cost.

The land and water requirements for sorghum present major problems in countries with limited resources. Thus, alternative carbon sources should be considered that do not require land and have lower water requirements, such as crude glycerol. By comparison, to produce microalgae with lipid contents of 52.5–73.4% by dry weight, sweet sorghum should be added into the medium at a concentration of approximately 25–50 g/l [3,26]. Based on an estimated price of $0.48 per kg, the cost of using sweet sorghum as a carbon source in microalgal culture can thus be up to $0.024 per liter of culture medium. In addition, sweet sorghum also serves as an excellent feedstock for ethanol production [53,246].

Crude glycerol, conversely, can be supplied from various sources. One economical source is a biodiesel refinery that processes waste vegetable oil from restaurants, in which one by-product is crude glycerol. This finding could make crude glycerol the most useful carbon source for microalgal culture for biofuel production. However, the limited quantity of glycerol waste available from biodiesel refineries would most likely make it an inadequate source for long-term cultivation. It was reported that the tolerable concentration of glycerol is within the range of 70–100 g/l [33], while varying the concentration from 1 to 12% was capable of increasing the lipid content up to 73.3% and increasing the biomass yield of microalgae [3,31,54]. The price of crude glycerol from biodiesel refineries and other sources is reportedly falling; according to Miller-Klein Associates [247], the current price of crude glycerol in the US is $0–70 per ton or $0.07 per kg, and some producers pay only for its transport to a purification unit. Thus, the production of microalgae with a lipid content of 73.3%, using approximately 35 g/l of crude glycerol, would incur an equivalent cost of $0.00245 per liter for the culture medium. Based on this mathematical evaluation, waste crude glycerol could be profitably used as a carbon feedstock in biofuel production, specifically in biodiesel from microalgae. However, this summary is based on current economics that will likely change over time.

The credit for using industrial flue gas to feed phototrophic microalgae can also be considered in the cost–benefit analysis. Most studies have shown that the tolerable concentration of CO_2-enriched air for microalgal growth is from 2 to 10% [14,37,248,249]. The CO_2-abatement benefit of using industrial flue gas in microalgae culture is as important as the cost reduction in the carbon source, as utilizing industrial flue gas also reduces carbon emissions and, hence, ameliorates global warming. Overall, the phototrophic culture of microalgae reduces the capital cost of microalgal cultivation.

9.3. The culture system

Production costs are the most important issue for algae biofuel commercialization. Therefore, this section will analyze and compared the capital and production costs of open pond and photobioreactors that have been reported in the literature. To obtain 40% extractable lipids from biomass, the annual microalgal productivity was 67 mt/ha/yr. By using flue gas as a carbon source, the operation cost can be reduced and the crude oil produced was $127 per barrel [31]. With this cost, the prices of biodiesel, gasoline and ethanol were $1.65, $1.70 and $2.75 per gallon, respectively, with the biomass feedstock production costs estimated at $211 per metric ton.

The most recent complete report on the analysis of algal production was reported in Seed Science by Alabi et al. [32]. The total production cost of algal oil was $14.44 per liter. The main source of this production cost was capital, labor, power and fertilizer, where the costs were approximately 49, 27 and 25% of the total, respectively. If the microalgal biomass lipid contents were 15%, the cost of production of algae would be $2.66 per kg. Both of the analyses conducted by Sheehan et al. [31] and Alabi et al. [32] show that the cost for microalgae as a feedstock for biofuel production is high. However, these analyses were based on assumptions that the lipid production of microalgae can be as low as 15%. If microalgae can be cultivated and produced with lipid contents as high as 73.4% as reported in literature [54], the production costs would be lower. In contrast, an energy balance of algae in an open pond culture, shows that for every 1 unit of energy input, approximately 1.76 units of energy is gained. This shows that microalgal cultivation in open ponds is possible for commercial purposes.

According to an economic feasibility study conducted by Seed Science Ltd., the total cost of every 5 l of oil produced using a photobioreactor, open pond and fermenter is 24.60, 14.44 and $2.58, respectively, if the microalgal cost is $7.32 for every 1 kg of dry biomass [165]. This price is high compared to petroleum fuels on the market. However, the energy balance conducted by Seed Science Ltd shows that for every 1 unit of energy consumed, approximately 1.23 units of energy is gained. This feasibility analysis shows that microalgal cultivation in a photobioreactor is profitable even though it is less competitive compared to other biofuel feedstocks. However, most studies have suggested that the cultivation of algae in bioreactors is most likely the most promising biofuel feedstock, but only if efforts are taken to reduce the capital costs.

10. Conclusion

Microalgae are feasible as a biofuel feedstock by taking the cultivation technique into consideration. By cultivating microalgae based on the two-stage growth period, high biomass production and the desired lipid productivity can be achieved. The credit for using flue gas to culture microalgae under phototrophic condition can be accounted in the cost benefit analysis of microalgal commercialization. However, the use of a switchable solvent for the extraction of lipid oil from the microalgae reduces the production cost because it can be reused and is easy to separate from the biomass. In addition to biofuel, microalgae also offer other benefits in pharmaceutics and wastewater treatment, therefore making microalgae one of the most useful organisms to humans.

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